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(*Recognosce notum, ignotum inspice*)

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AQUATIC HYPHOMYCETES FROM JAMAICA

By H. J. HUDSON
University College of the West Indies
and C. T. INGOLD
Birkbeck College, University of London

(With Plate 11 and 7 Text-figures)

Aquatic hyphomycetes have been collected from fifteen streams in Jamaica. Fourteen known species are recorded. *Jaculispora submersa* n.gen., n.sp. is described. Certain spore types belonging to unknown species are illustrated.

In March 1959 we made a brief survey of aquatic hyphomycetes on leaves of dicotyledonous trees decaying in streams in various parts of Jamaica. March is in the middle of the dry season, so the streams examined were the relatively few permanent ones mainly in the more mountainous regions of the island. They were mostly small and shallow with rapidly flowing water ensuring good aeration.

Usually a sample of brown, rotting leaves (chiefly petioles) was collected, thoroughly washed and aerated in water for a day or two before examination. Scum and foam were only rarely encountered on the surface of the streams, but when found samples were collected and examined for trapped spores. The most rewarding collections of this nature came from a beautiful beck (Buff Bay River) near the famous Hardwar Gap.

In Table 1 a list is given of the fungi found in the various streams. Seven species are particularly common, occurring in nine or more of the fifteen streams sampled. Of these species *Lunulospora curvula* Ing., *Flagellospora curvula* Ing. and *Tetracladium marchalianum* de Wild. are also very abundant British species.

Triscelophorus monosporus Ing., so common in Jamaica that it was found, usually in quantity, in all the streams, is known in Britain only from the type collection, but spores have been seen in abundance in scum samples from W. Africa (Ingold, 1956) and E. and Central Africa (Ingold, 1958). The fungus has also been found in California (Ranzoni, 1953) and Japan (Tubaki, 1957).

Campylospora chaetocladia Ranzoni (1953), described from California, is now known in E. Africa (Ingold, 1958) and W. Africa (Ingold, 1956). It does not appear to be a British species, though spores of what seems to be another species had been figured from Britain before the publication of Ranzoni's paper (Ingold & Ellis, 1952). It is very common in Jamaica, occurring in miniature forests on many leaves from nearly all the streams.

Heliscus tentaculus Umphlett (1959), described from Virginia, U.S.A., had

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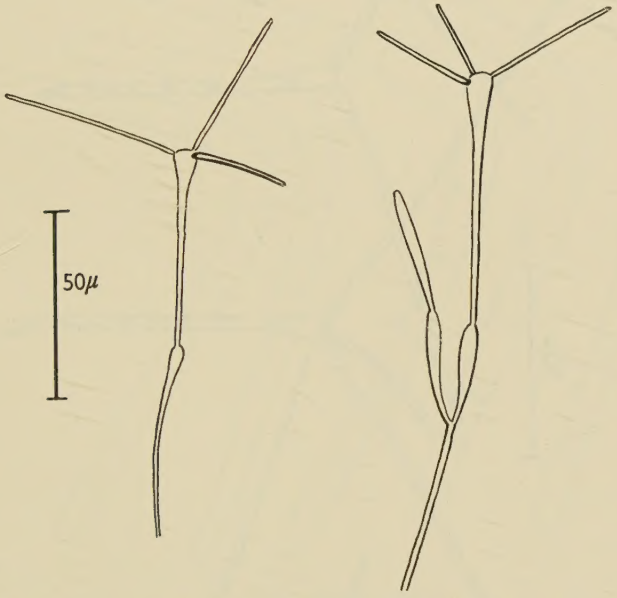
Table 1. *Aquatic hyphomycetes found in Jamaica*

Species	Black R. (Maggoty)	Milk R. (Porus)	Clyde R.	Wag Water R. (Grandy Hole) (trib.)	Wag Water R. (Stony Hill) (trib.)	Stony R.	Dunn's R.	Negro R.	Shooting R. (Ramble)	Banana R.	Buff Bay R. (Hardwar Gap)	Hope R. (trib.)	Fall R. (Mahogany Vale)	Hog Hole R. (trib.)	Hog Hole R. (main)
Triscelophorus monosporus	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗	⊗
Campylospora chaetocladia	×	⊗	⊗	⊗	⊗	⊗	·	⊗	×	⊗	⊗	⊗	⊗	⊗	⊗
Flagellospora penicillioides	⊗	⊗	⊗	·	·	·	⊗	·	⊗	·	·	·	·	·	·
Lunulospora curvula	⊗	⊗	⊗	⊗	⊗	⊗	⊗	·	⊗	⊗	⊗	·	·	·	·
Heliscus tentaculus	·	·	×	·	·	·	·	·	·	·	⊗	·	·	·	·
F. curvula	·	·	⊗	·	·	·	·	·	·	·	⊗	·	·	·	·
Tetracladium marchalianum	·	·	⊗	·	·	·	·	·	·	·	⊗	·	·	·	·
Actinospora megalospora	·	·	·	·	·	·	·	·	·	·	×	·	·	·	·
Alatospora acuminata	·	·	·	·	·	·	·	·	⊗	·	⊗	·	·	·	·
Anguillospora crassa	⊗	·	·	·	·	·	·	·	·	·	⊗	·	·	·	·
A. longissima	·	·	·	·	·	·	·	·	·	·	⊗	·	·	·	·
Articulospora tetracladia	⊗	·	·	·	·	·	·	·	·	·	⊗	·	⊗	·	·
Clavariopsis aquatica	·	·	·	·	·	·	·	·	·	·	⊗	·	·	·	·
T. setigerum	·	·	·	·	·	·	·	·	·	·	⊗	·	·	·	·
Heliscus (?) sp.	·	·	·	·	·	·	·	·	×	·	·	·	·	·	·
Jaculispora submersa	·	·	·	⊗	⊗	·	·	⊗	·	·	·	·	·	·	·

⊗ indicates conidiophores attached to leaves. × indicates spores alone.

previously been seen as spores both in Uganda and in Southern Rhodesia (Ingold, 1958). It was common in Jamaica and all stages of development were observed. There was usually only one phialide terminating the rather long conidiophore, but occasionally two occurred (Text-fig. 1). This is the largest and most elegant of the species of *Heliscus* yet described, and its discovery reinforces the case for erecting a new genus for this species, together with *H. longibrachiatus* Ing. and *H. stellatus* Ing. & Cox (1957).

Nectria (*Flagellospora*) *penicillioides* Ranzoni was described (Ingold, 1944) from a single isolation in Britain and has not been reported from there since. Ranzoni (1953), however, found it in California and has succeeded (Ranzoni, 1956) in producing its *Nectria* state in culture. Further Dr P. Kable sent one of us (C.T.I.) a culture of the fungus from Australia. In Jamaica it occurred frequently in the 'imperfect' state.

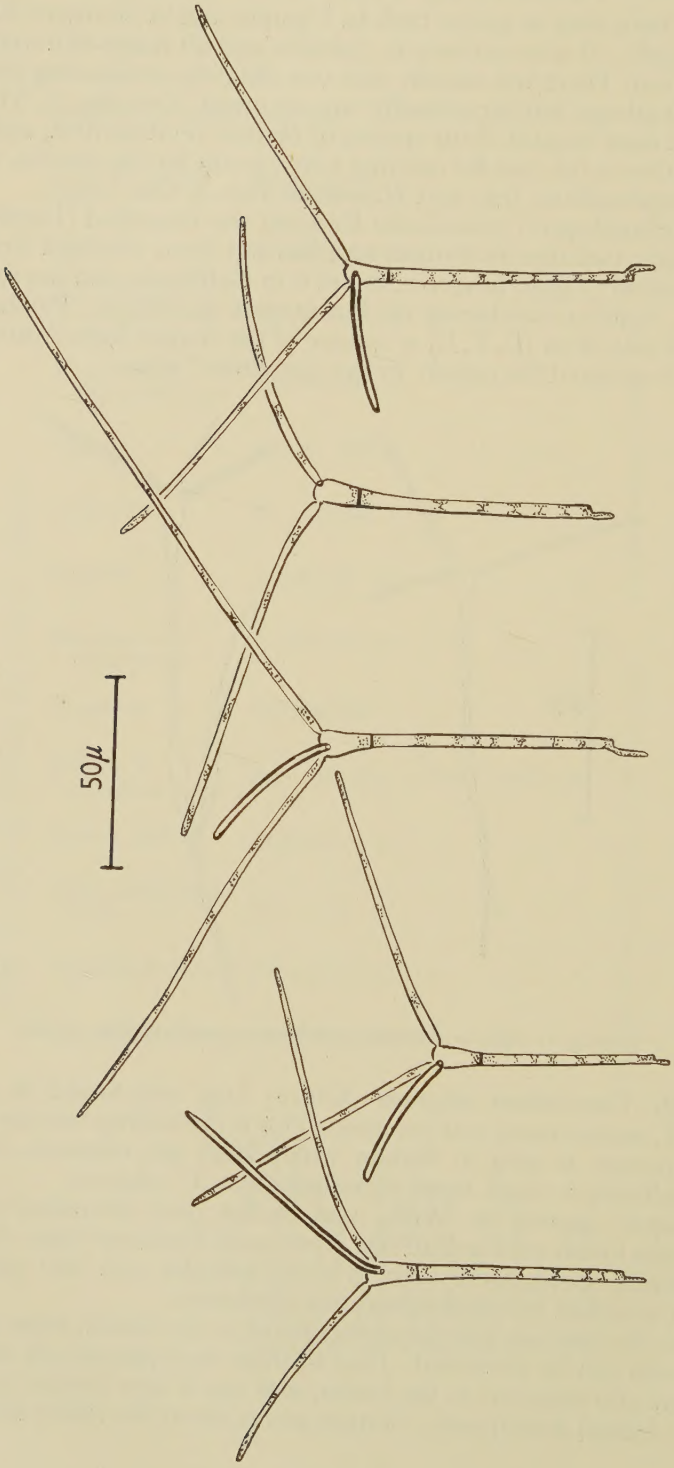


Text-fig. 1. *Heliscus tentaculus*, conidiophores with conidia. × 500.

Though *Tetracladium setigerum* (Grove) Ing. was found in only one stream, *T. marchalianum* was common. This is interesting because the same kind of picture is seen in Britain where both are common, but again *T. marchalianum* is much more abundant than *T. setigerum*.

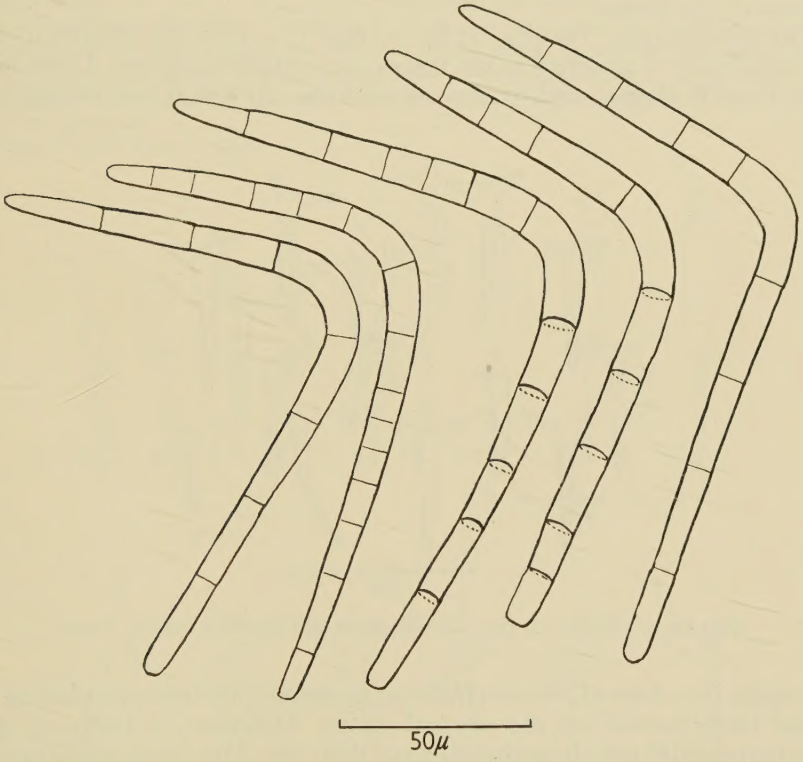
Clavariopsis aquatica de Wild., one of the most abundant species in Britain, was found only in Buff Bay River near Hardwar Gap. A few of its characteristic spores were seen in scum samples and two spores were observed attached to conidiophores on the leaves.

Besides the fourteen known species found in the island, some were seen which could not be identified. Four of these were present not only as free spores but also attached to the leaves, and one is now known in sufficient detail for formal description. Certain points about the others need further



Text-fig. 2. *Clavariopsis* sp., liberated spores. × 500.

study. One of these (Text-fig. 2) is known to us mainly as liberated spores, and many were examined. Further, some attached spores were seen and their development was watched. However, the details of attachment to the conidiophore were not clearly seen, largely because the specimens observed were on rather thick petioles and also because the conidiophores appeared to be very short. However, we have little doubt that this particular fungus is a species of *Clavariopsis*. The spore consists of four long divergent arms, and of these the first-formed is 55–75μ long by 4μ wide.



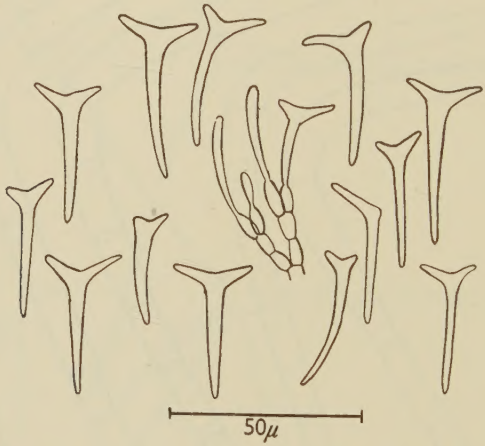
Text-fig. 3. *Anguillospora* sp., spores from stream near Hardwar Gap. × 500.

There is constantly a single septum 10–15μ behind the slightly swollen crown of this arm. From this crown three arms (60–140μ long by 2μ wide) diverge, each being slightly constricted at its origin. The spore on liberation is vacuolate, this being especially evident in the first-formed arm. A curious feature, which serves to make these spores particularly characteristic, is a small projection (4–8 × 1·5μ) from the base of the first-formed arm which always arises on one side of the basal detachment scar. At first it was thought that spores with this feature represented specimens which had started to germinate, but it was soon realized that the projection was present immediately after spore liberation, and further it did not continue to elongate in spores suspended in water. No doubt it could be inter-

preted as an arrested germ-tube, but it seems more likely that it functions in some way in relation to spore detachment.

A second fungus, known both from attached and detached spores, clearly belongs to *Anguillospora*. *A. longissima* Ing. and *A. crassa* Ing. were both identified and this other species was found as isolated spores from Buff Bay River near Hardwar Gap and also attached in leaf collections from two other localities. The hyaline, septate spore is always boomerang-shaped, 8–10 × 200–250 μ (Text-fig. 3; Pl. 11, fig. 5) and never sigmoid as in the other species. The base and apex are also clearly distinguishable, an unusual feature in the genus.

The third fungus (Text-fig. 4; Pl. 11, figs. 1, 2) was seen frequently both on conidiophores attached to the leaves and as isolated spores. These spores were T- or Y- shaped and mostly non-septate. At first it was thought that



Text-fig. 4. *Heliscus* (?) sp., a conidiophore and liberated spores. × 500.

this might be a form of *Nectria* (*Heliscus*) *lugdunensis* Webster, producing two apical teeth instead of the normal three. However, a study of spore development did not altogether support this view. The young phialospore is straight or more usually slightly curved, increasing by apical growth. Finally, the original apex is somewhat arrested and a new growing point makes its appearance a little way behind the original one and grows out laterally. Very recently one of us (H.J.H.) has isolated this in pure culture. The spores produced in sticky heads on agar and also those formed on strips of colony in aerated water show a still stronger resemblance to those of *N. lugdunensis* except in the presence of only two apical projections. Further, a single septum is normally present just beyond half way along the spore. It is hoped to report more fully on this species later.

Jaculispora Hudson & Ingold gen.nov. (Latin *jaculum*, a dart)

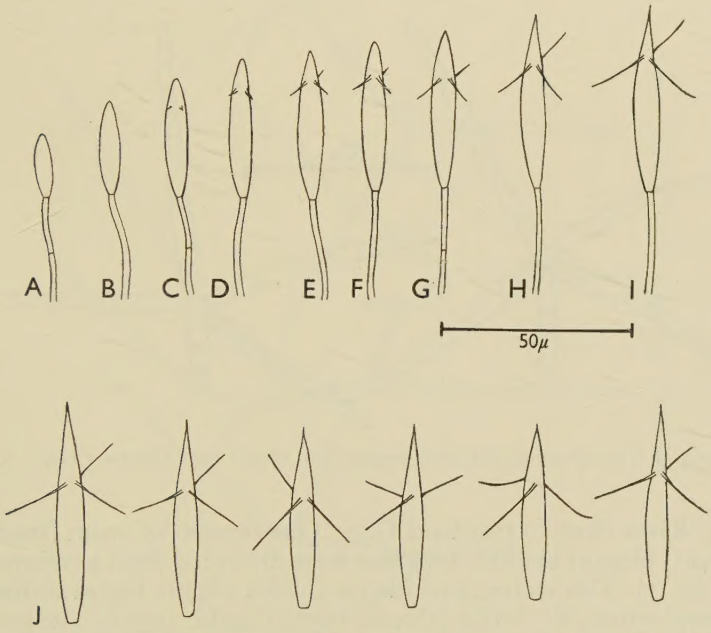
Fungi aquatici submersi, mycelio septato, hyalino. Conidium (thallospora terminalis) hyalinum, unicellulare, consistens ex longo et crasso principali axe ad apicem attenuato ferente tres tenuissimos ramulos orientes ex superiore parte. Typus: *J. submersa.*

Jaculispora submersa Hudson & Ingold sp.nov. (Pl. 11, figs. 3, 4; Text-fig. 5)

Fungus aquaticus submersus, mycelio septato. Conidiophora hyalina, simplex (aut pauculis ramis), 20–120 μ longa, 1·5–3 μ lata, cum inconspicuis septis, unum conidium ferens. Conidium (thallospora terminalis) hyalinum, unicellulare, consistens ex crasso axe principali (37–55 μ longo, 5–7 μ lato in media parte) ad apicem attenuato, ferente tres tenuissimos ramulos (10–24 μ longos, 0·5–1 μ latos) orientes ex superiore parte.

Habitat. In foliis putrescentibus ex rivulo influente Wag Water River ad Grandy Hole, St Mary, Jamaica.

Typus: Herb. I.M.I. 76791.



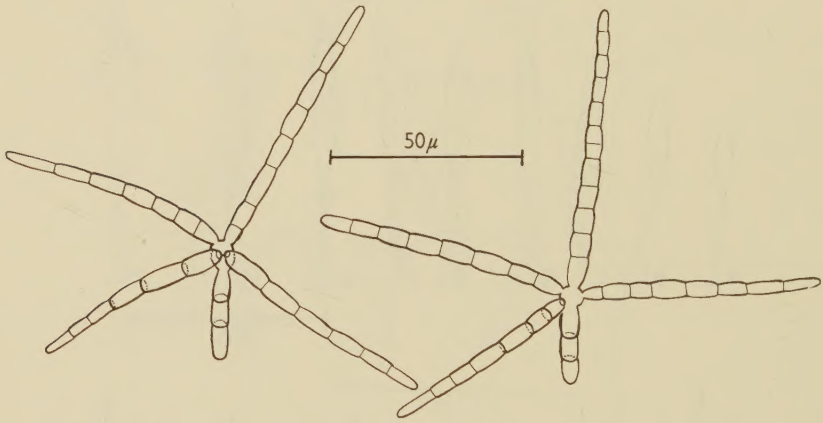
Text-fig. 5. *Jaculispora submersa*: A–I, conidiophores with conidia at various stages arranged in a developmental series; J, liberated conidia. × 500.

This species was found growing on leaves from three streams and as detached spores in a fourth. All stages in spore development were observed.

The mycelium within the leaf gives rise to simple or sparingly branched, very narrow and delicate conidiophores, 20–120μ long, 1·5–3μ wide with inconspicuous cross-walls. During spore development the tip of the conidiophore becomes swollen and club-shaped. The spore primordium soon becomes cut off from the conidiophore by a transverse septum, although this is not easily demonstrable. As the spore develops, the apex

becomes increasingly pointed and three very fine diverging hair-like lateral appendages arise and grow out simultaneously. These are equally spaced around the circumference and arise at a level just beyond half way between the base and apex (Text-fig. 5*c–h*). When mature the spore breaks away from the conidiophore at the septum. It is thus a terminal thallospore. It is hyaline, apparently aseptate and consists of a relatively substantial straight main axis 35–55 μ long with a truncate base. It is 3–3·5 μ wide at the base increasing to 5–7 μ wide at a distance of about 25–35 μ from the base. It then gradually tapers to the apex ending in a fine point, making the spore appear dart-shaped except for the three lateral appendages which are 10–24 μ long and 0·5–1 μ wide.

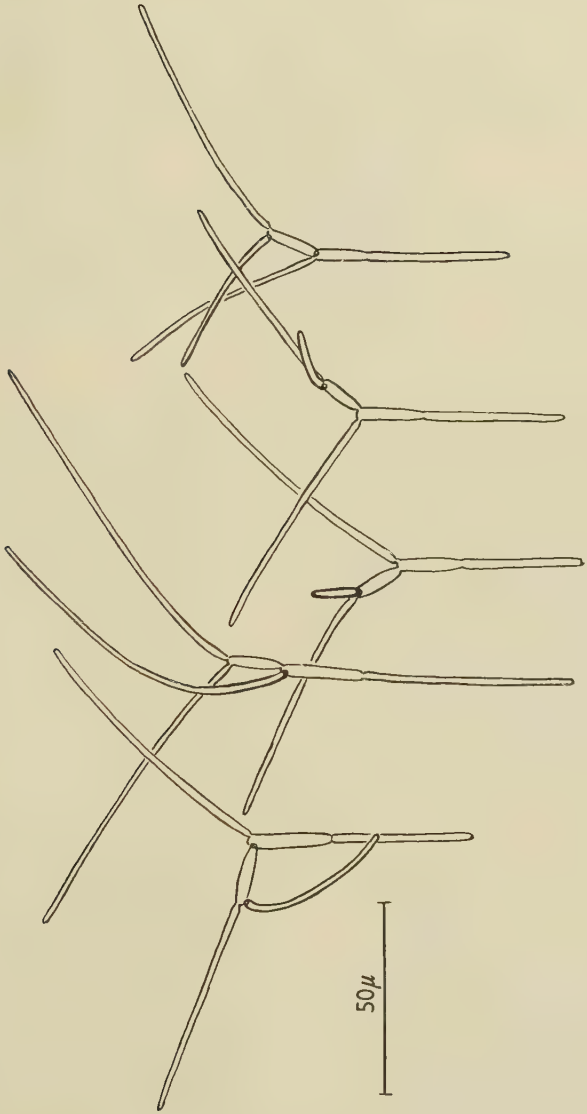
A number of kinds of detached spore were seen which seem to belong to undescribed fungi and some of the commonest of these deserve brief mention. Two of them were particularly abundant in collections from the



Text-fig. 6. Two spores of unknown fungus from stream near Hardwar Gap. × 500.

Buff Bay River close to Hardwar Gap. One consists of seven (more rarely six or eight) elegant hyaline, tapering arms diverging from a common point (Pl. 11, fig. 7). This seems to be identical with a spore found commonly in scum samples from W. Africa (Dixon, 1959; Ingold, 1959). The second has a relatively short main axis (5 × 30 μ) with a slightly inflated head from which four (more rarely three or five) arms (5 × 60–80 μ) diverge (Text-fig. 6). All the branches are hyaline, conspicuously septate and have cross-walls rather close together with slight constrictions at many of them. Further, the walls are not as delicate as in most aquatic hyphomycetes. The cells are non-vacuolate and the contents highly refractive. This spore invites comparison with one (Ingold, 1958, fig. 1) already described from E. Africa.

Another hyaline spore type found in large numbers associated with decaying leaves from four different streams is shown in Text-fig. 7. It is large and delicate with inconspicuous septa and may possibly belong to a species of *Tricladium*.



Text-fig. 7. *Tricladium* (?) sp., spores. ×500.

Only two specimens of the type of spore shown in Pl. 11, fig. 6 were seen, both from the stream near Hardwar Gap. This is very like the spores found very occasionally in Britain (Ingold, 1958) and Nigeria (Ingold, 1959). However, in no locality has attachment to the conidiophore yet been observed.

Our thanks are due to Prof. E. H. Warmington for his help with the Latin diagnoses.

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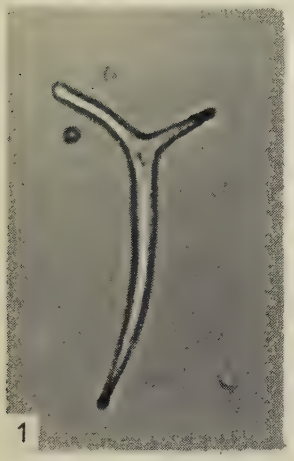
EXPLANATION OF PLATE 11

Figs. 1, 2. *Heliscus* (?) sp. spores. × 1000.
Figs. 3, 4. *Jaculispora submersa* spores. × 1000.
Fig. 5. *Anguillospora* sp. spores. × 500.
Fig. 6. Spore of unknown fungus. × 1000.
Fig. 7. Spore of unknown fungus. × 500.

(*Accepted for publication* 23 *July* 1959)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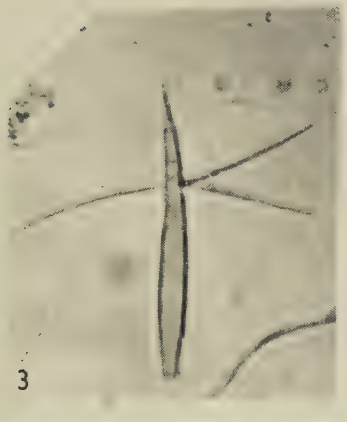
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Trans. Brit. mycol. Soc. **43** (3), 479–488 (1960).

A CRITICAL STUDY OF THE TAXONOMY OF THE *ACHLYA SPINOSA, A. STELLATA, A. CORNUTA* COMPLEX*

By M. W. DICK

Queen Mary College, University of London

(With Plate 12 and 4 Text-figures)

Achlya spinosa de Bary and *A. stellata* de Bary are redescribed and illustrated; a critical account is given of their taxonomy together with that of *A. cornuta* Archer which is placed in synonymy with *A. stellata.*

Johnson, in his monograph (1956) of *Achlya*, was unable to arrive at any conclusion concerning the validity of the species *A. spinosa* de Bary (1881), *A. stellata* de Bary (1888) and *A. cornuta* Archer (1867), and relegated them to the category of doubtful species. Two of them have recently been isolated from soil on Wimbledon Common, Surrey, and elsewhere. The following descriptions are based upon unihyphal cultures grown on boiled hemp seed in sterile, glass-distilled water at 18–20° C. Measurements of oogonia and oospores are from 300 oogonia in a number of different cultures and those of zoosporangia from 100 for each species. Many additional oogonia and zoosporangia were examined.

I have examined de Bary's original specimens (slides) of *A. spinosa* and *A. stellata* in the British Museum (Natural History) and they are clearly the same as the two fungi I have found and redescribed here. The characteristic gemmae of *A. spinosa* may be seen in de Bary's preparations and also chains of up to three intercalary oogonia. His slide of *A. stellata* shows a number of sporangia similar to those described below, also clusters of zoospore cysts.

Achlya spinosa de Bary

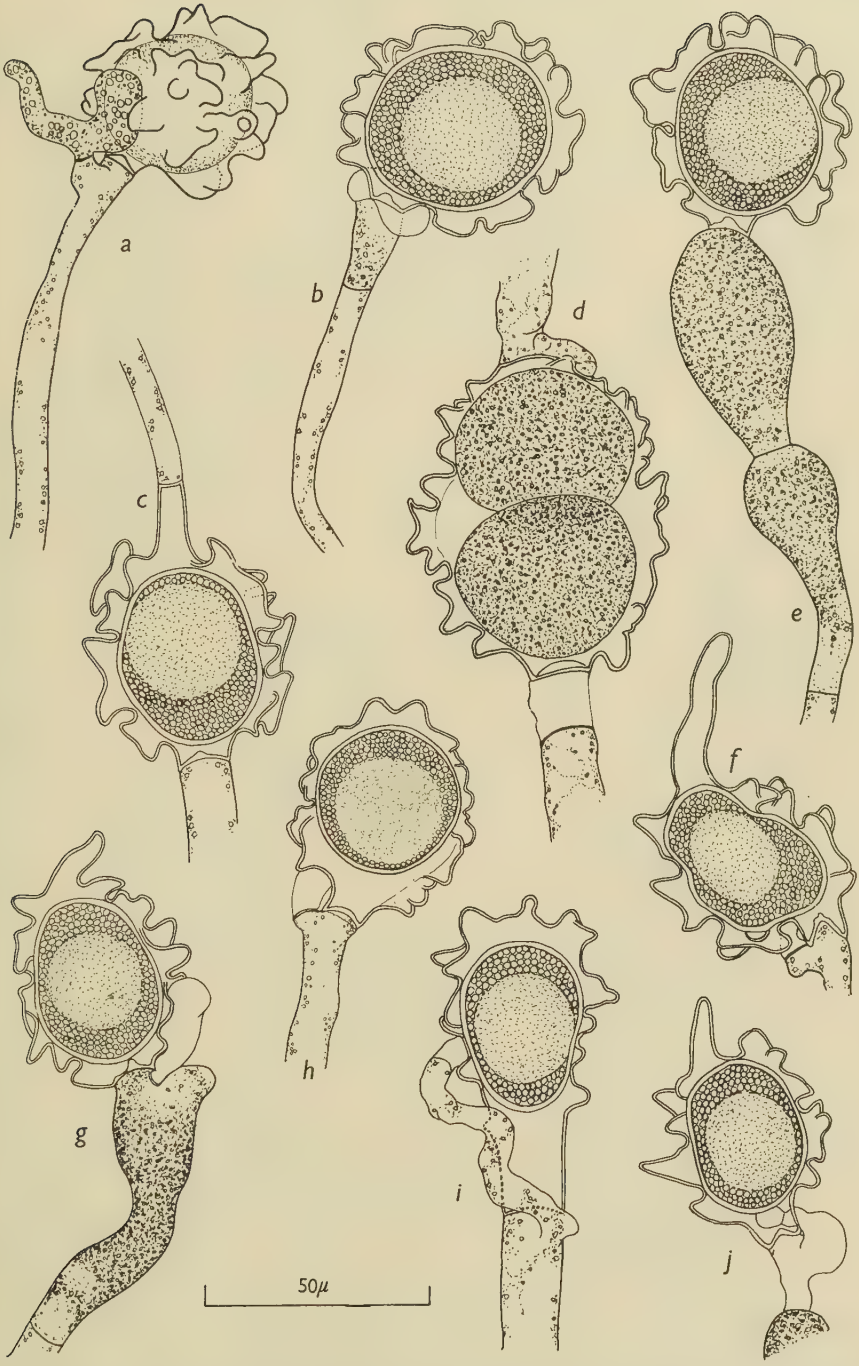
Mycelium extensive, 3·5–4 cm. diam. after 2 weeks, resembling a tuft of pure white sheep's wool, owing to the twisted vegetative hyphae and numerous dense gemmae. *Hyphae* approximately 60–90 μ diam. near the base, elsewhere the majority are approximately 30 μ diam., sparingly branched. *Zoosporangia* infrequent (about 100 on a single 'turf'), filiform or very long-clavate, relatively thin walled, but with a thickened exit papilla; renewal lateral or basipetalous, occasionally internal; secondary sporangia often longer; basal septum is usually distinctly domed (Text-figs. 3, *g*; 4, *i*); dimensions in the range (258) 408–714 (1666) μ by (20) 26 (41) μ. *Zoospores* typical for the genus except that the cyst cluster is unstable, separating within a few minutes; dehiscence occasionally dictyoid or aplanoid. *Gemmae* abundant, either (*a*) long, spindle-shaped, usually intercalary,

* Part of a thesis approved for the degree of Ph.D. in the University of London.

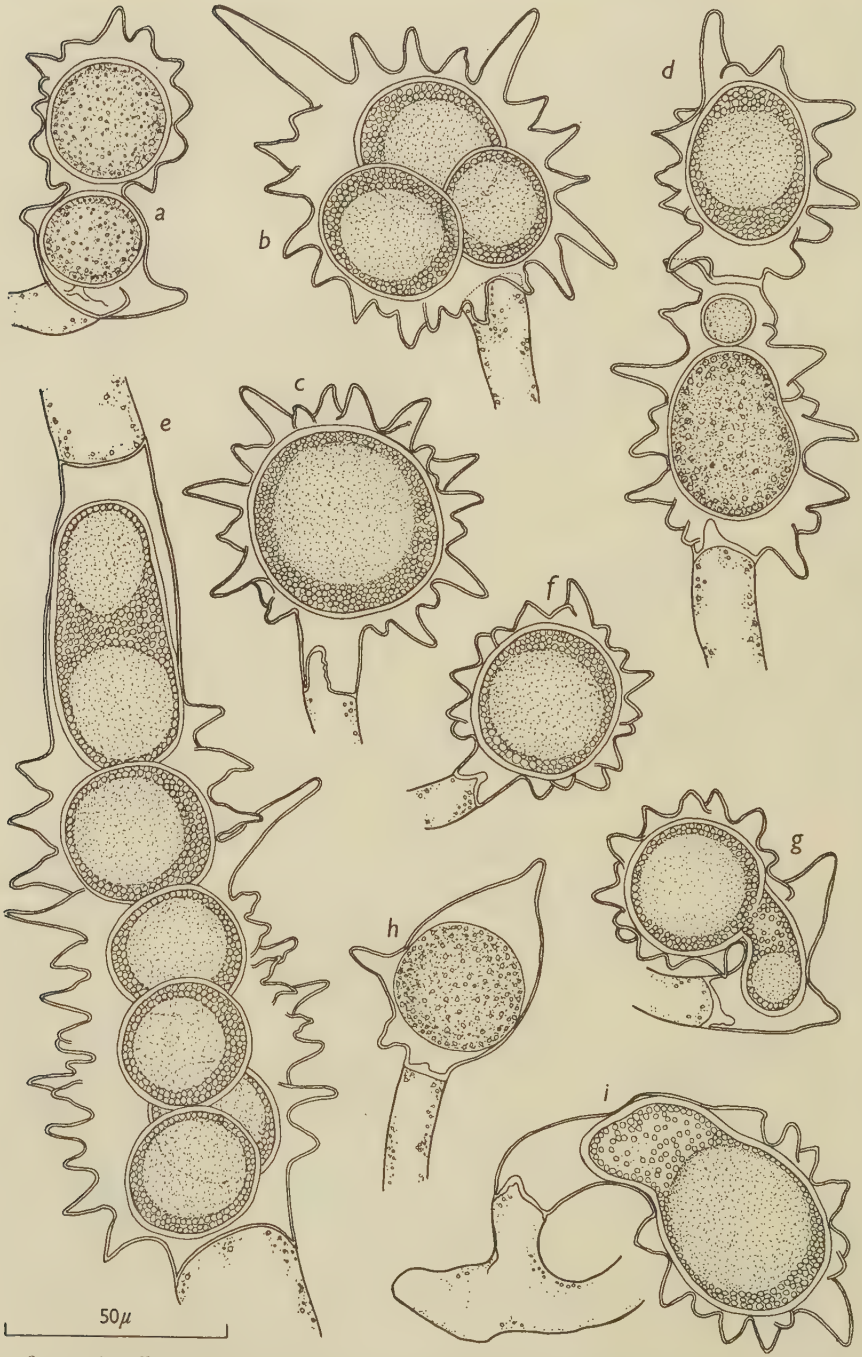
often with a curved longitudinal axis; or (*b*) short, clavate, usually terminal or subterminal, occasionally beneath oogonia; chains of either type may occur, or mixed chains in which there are normally 1–2 long gemmae with a few clavate gemmae at one or both ends; germinate readily in fresh glass-distilled water or dilute soil extract, usually by one or two short, slender hyphae from one end, the whole structure then behaving as a zoosporangium with a typical exit papilla (Text-fig. 3, *d*, *e*); zoospores are arranged in single file in the hyphal outgrowth. *Oogonia* often infrequent in rough culture and always so in pure culture, often entirely absent; single in rather old (3-week) cultures on variable, but usually long (up to 600 μ and frequently over 400 μ), narrow (10–15 μ), undulating lateral branches; or intercalary, when they are usually single, but rarely in chains or with intercalary gemmae (Pl. 12, fig. 1 top right); spherical, ovoid or doliform, wall densely ornamented with blunt papillate, lobed or wart-like projections, often (e.g. 32 %) with a terminal apiculus; pits absent; diam. in the range (30) 47–59 (103) μ, including ornamentations. *Antheridia* usually present (de Bary suggests on only about 50 % of the oogonia); when present normally one to an oogonium, androgynous (monoclinous) on a very short antheridial branch or semi-hypogynous (very rarely diclinous according to de Bary): antheridial cells thin walled, clavate, laterally applied to the oogonium between the papillations. Fertilization tubes observed by de Bary but not seen with certainty in my isolates. *Oospheres* usually single (over 90 %) or rarely up to 4 in an oogonium, maturing, and filling the oogonium (apart from the papillae), frequently (50 %) more or less ovoid. *Oospores* subcentric, cytoplasm occasionally incompletely surrounded by the oil droplets; diam. (19) 30–37 (44) μ. Germination not observed (but resting period 8–10 days according to Fischer).

ACHLYA STELLATA de Bary

Mycelium restricted (2-week-old turf about 2·5 cm. diam.). *Hyphae* approximately 60–70 μ diam. near the base, elsewhere approximately 25–40 μ diam., sparingly branched. *Zoosporangia* moderately abundant (some authors suggest that they are sparse), fusiform, less frequently naviculate or filiform, (210) 354–490 (782) μ by (20) 39 (54) μ. *Zoospores* typical for the genus, cyst cluster not persistent. *Gemmae* absent. *Oogonia* abundant, terminal on main hyphae or on lateral branches which are commonly curved; very rarely intercalary and extremely rarely in chains; often in a dendroid arrangement at the end of a principal hypha, less frequently more or less racemose. Oogonia spherical or less frequently longer than broad, often with a neck; the basal septum often having an inward projection; wall densely ornamented with conical or mammiform papillae, rarely almost smooth. (In a variable number (but often less than 10 %) a terminal apiculus is evident. The curved axis of the oogonial stalk is frequently continued by the apiculus. A feature is for the oogonium to be formed laterally near the extreme tip of the hypha, leaving a large conical projection near the basal septum (Text-fig. 2, *a*, *g*); this is probably a marked manifestation of the tendency to a curved oogonial axis.) Pits absent; diam. in the range (34) 48–61 (94) μ inclusive of ornamentations.

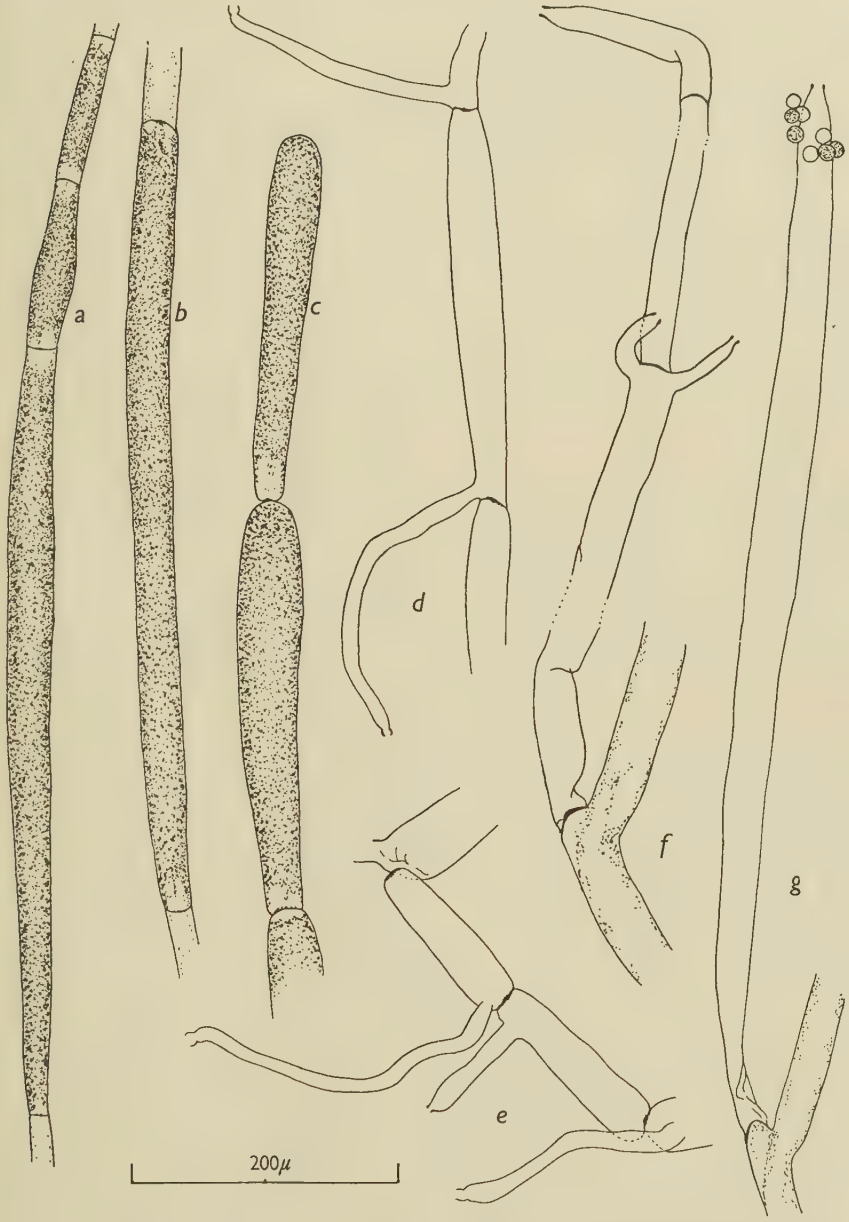


Text-fig. 1. *A. spinosa.* Oogonia: *a*, surface view showing ornamentations and androgynous (monoclinous) antheridium; *a, b, e–h, i*, lateral oogonia; *c, d*, intercalary; *e, g, j*, oogonia with gemmae; *f, g, j*, with an apiculus; *b, d, j*, with a semi-hypogynous antheridium (possibly a fertilization tube in *j*); *f, i*, with an androgynous (monoclinous) antheridium; *c, e*, no antheridia; *c, e, i*, with extreme subcentric oospore structure.



Text-fig. 2. *A. stellata*. Oogonia: *f*, typical; *b*, *c*, largest lateral oogonia observed; *d*, two oogonia in a chain; *e*, intercalary oogonium (note similarity in shape to those of *A. radiosa*); *f*, *i*, *g*, *a*, a series showing the characteristic curving of the oogonial axis, with cone-like projections from the base of the oogonium in *g* and *a*, and also a tendency to form two oospores; *h*, an almost smooth oogonium with an apiculus. Note the sparseness of oil droplets in parts of the oospores in *g* and *i*.

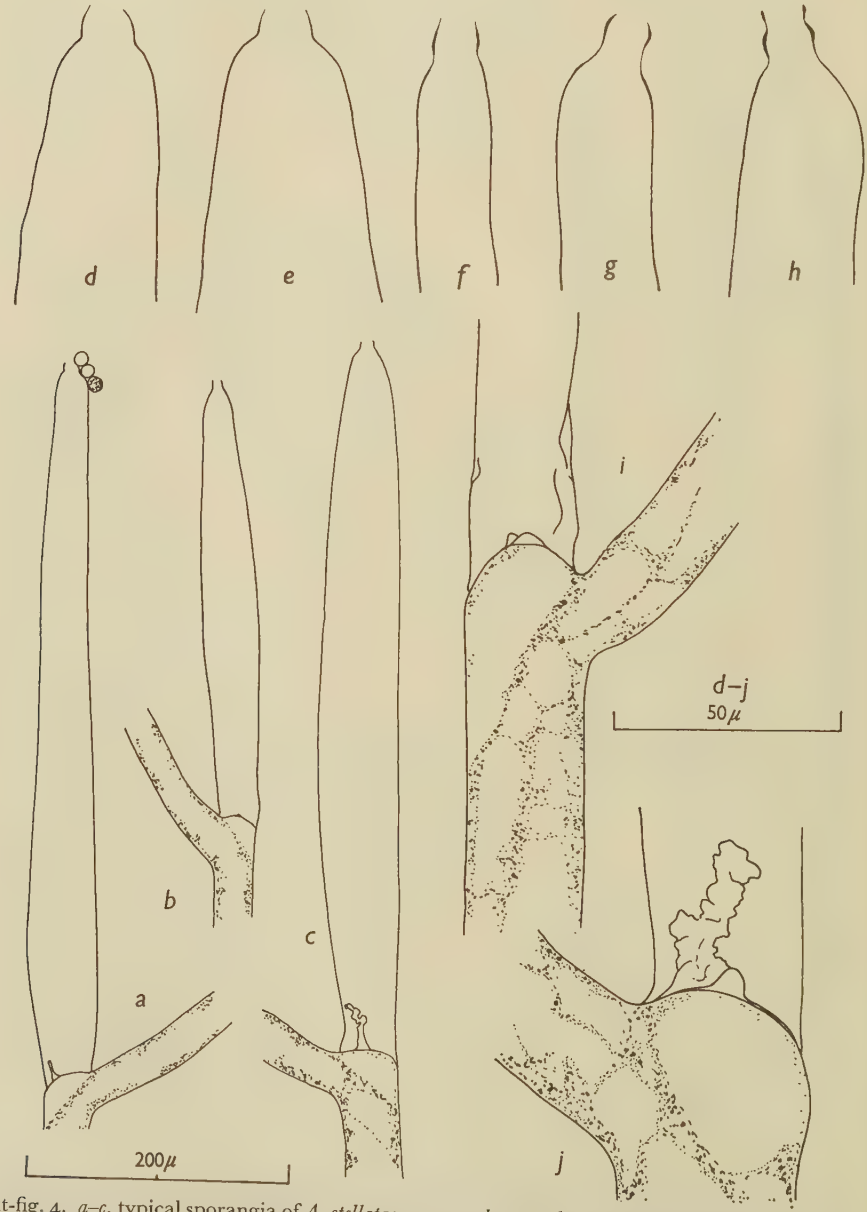
Antheridia absent. *Oospheres* normally one (about 95 %) but up to six in an oogonium, normally spherical, but occasionally (less than 20 %) ovoid or irregular, filling the oogonium (apart from papillae) and maturing.



Text-fig. 3. *A. spinosa*. *a–e*, gemmae: *a*, *b*, *d*, long; *c*, *e*, short; *d*, *e*, have germinated and behaved as sporangia; *f*, a chain of sporangia, the lowest 1258 μ, the middle (papilla not shown) 680 μ and the terminal 159 μ long; *g*, sporangium 790 μ long, with high domed basal septum.

Oospores subcentric, oil droplets in some parts sparse and apparently embedded in a gel surrounding the more fluid cytoplasm. Diam. (20) 29–35 (44) μ. Germination not observed.

A discussion of the taxonomy of these two species must also include *A. cornuta* Archer, since these three species have been regarded as forming a



Text-fig. 4. *a–c*, typical sporangia of *A. stellata*: *a*, 549 μ, *b*, 337 μ long; *d*, *e*, apical papillae; *j*, base of sporangium; *f–h*, apical papillae showing thickening; *i*, *A. spinosa*: base of sporangium.

complex, which Johnson (1956) considered should be discussed as a unit. The following description of *A. cornuta* has been compiled from Archer (1867), Humphrey as quoted by Coker (1923) and Johnson (1956) and Minden (1912).

ACHLYA CORNUTA Archer

Hyphae of medium size, short. *Zoosporangia* rare, cylindrical. *Oogonia* terminal, either single or in series on principal hyphae, or on mostly short, straight or arched, lateral branches, racemosely arranged; globular or ellipsoid, densely set with rather long, cylindrical or bluntly conical papillae; pits absent; apiculus often evident. *Antheridia* absent. *Oospores* 1–4 (10) globular or slightly elongate, centric; diam. about 29 μ.

None of these has been described more than four or five times and no author appears to have seen all three. De Bary (1888), Minden (1912), Ramsbottom (1916) and Höhnk (1935) observed two of them, but neither Ramsbottom nor Höhnk gave descriptions or illustrations of either. De Bary records *A. spinosa* and *A. stellata*; Minden records *A. stellata* and *A. cornuta*, but also gives a description of *A. spinosa*; Höhnk and Ramsbottom both record *A. spinosa* and *A. cornuta*. The descriptions given by Fischer (1892) and Migula (1903) appear in the main to be transcriptions of de Bary's original diagnoses.

Johnson considered *A. stellata* to be the most concisely described of these species. It was tentatively suggested by Humphrey, and more definitely stated by Coker, to be synonymous with *A. cornuta*. The main difficulties in the way of accepting this proposal are: (*a*) the characteristics of the terminal oogonia, (*b*) the number of oospores, (*c*) the sporangia, and (*d*) the fact that Minden thought he found both species and regarded them as distinct. It is proposed to consider the characters in relation to the present study of *A. stellata*.

Oogonia and oospores

In my cultures oogonia were mainly terminal on curving lateral branches or on main hyphae and very rarely intercalary or in chains. In approximately 95 % of oogonia I found a single oospore, but up to six can occur in an oogonium (Text-fig. 2, *e*). Early descriptions of *A. stellata* mention only terminal, globular or ellipsoid oogonia with one oospore. This may be compared with descriptions of *A. cornuta*: from Archer's description, chains of oogonia are apparently a prominent characteristic, and he reported the oospores to be '. . . perhaps as many as eight or ten, though ordinarily fewer'. Both Humphrey and Minden query the number of oospores reported by Archer, and neither regards the presence of chains of oogonia as important or conspicuous. Apinis (1930) described *A. stellata* and discounted the synonymy on the grounds that he did not observe such a range of variation in his isolate.

Sporangia

Details of sporangia in previous records are either lacking or sparse. For *A. stellata*, de Bary omits to mention sporangia and Apinis did not find them. Fischer and Migula describe them as resembling other *Achlya* species. By contrast, Minden describes them as sparse, *often remarkably long*,

cylindric, scarcely broader than the hypha. It will be recalled that long sporangia (10% > 1000μ long) are a characteristic of my isolate of *A. spinosa*. In my cultures of *A. stellata*, sporangia were moderately abundant, but otherwise would fit all descriptions except Minden's, as being not markedly different from other *Achlya* species. For *A. cornuta*, Archer failed to observe sporangia, Humphrey saw one but failed to describe it and Minden describes them as cylindric but scarce. It is only in the case of *A. stellata* sensu Minden that there is a stated difference in the shape of the sporangia.

A. stellata sensu Minden does not conform to other descriptions of *A. stellata* de Bary in other ways; Minden's is the only description to suggest long, rigid main hyphae, as against thin flaccid main hyphae, or the scarcity of oogonia in some cultures and the occurrence of blunt ornamentations on the oogonia. Johnson has also remarked on the similarity between *A. stellata* sensu Minden and *A. spinosa* de Bary. The difficulty in regarding these as synonymous lies in the absence of antheridia in Minden's fungus. This characteristic appears variable in *A. spinosa*, but owing to the origin and size of the antheridial branch and the thin nature of its walls, I have found that this structure may be very difficult to observe in old cultures or at low magnifications.

Regarding the intercalary oogonia of *A. spinosa*, there is a contradiction in de Bary's two descriptions (the only first-hand descriptions of this fungus), which has already been noted by Humphrey and Johnson. It is quite inexplicable because the later (1888) description appears incorrect in this respect.

The literature is also confused on the structure of the oospore in *A. spinosa*. De Bary may have had in mind the extreme type of subcentric structure (shown in Text-fig. 1, *c*, *e*, *i*) when he mentions and illustrates a large central fat globule surrounded by a complete or interrupted ring of globules; but this description was taken by Minden to refer to an eccentric oospore, though de Bary implied a distinction between this and a true eccentric structure (Johnson). One may discern here the reason for Minden's recognition of the three species and for his failure to realize that his *A. stellata* was probably no different from *A. spinosa*.

A further source of confusion is that de Bary noted the similarity (but also the distinctiveness) of *A. cornuta* and *A. spinosa* (1881) but thereafter (1888) made no mention of Archer's species, probably causing Fischer to treat them as synonymous.

The woolly snow-white turf of *A. spinosa*, mentioned in all descriptions, deserves comment. It is not (as Humphrey interprets) due to the production of 'very abundant, closely set, short-lateral branches', but rather to the many long, entwined, undulating principal hyphae and their branches. The remarkable opacity and whiteness comes from the very numerous, dense gemmae. There is little doubt that the sporangia of *A. spinosa* (described in 1881 and subsequently) with the zoospores often arranged one behind the other, are gemmae which had germinated.

It now seems reasonably certain that both Minden and Humphrey were describing as *A. cornuta* forms which would be indistinguishable from *A. stellata*. It is less easy to reduce to synonymy Archer's species. How-

ever, since his description was unfortunately based on material infected with a parasite, his species is in any case invalid. It is therefore proposed that *A. cornuta* Archer and *A. stellata* de Bary be regarded as conspecific, also that Minden's fungus was *A. spinosa*.

The confusion concerning *A. spinosa* and *A. stellata*, which exists in the literature, is understandable in view of a considerable superficial similarity between them, in respect of sexual organs; nevertheless their divergent habits, the difference in sporangial structure and the presence of well-defined gemmae in *A. spinosa* make it unlikely that these two species are closely related. The absence of antheridia in *A. stellata* and the distinct difference in the type of papillation of the oogonia are further useful points of distinction.

It is possible that *A. spinosa* has affinities with *A. treleaseana* (Humphrey) Kauffman, from which it differs mainly in the ornamentation of the oogonia and in the number of oospores; whereas *A. stellata* may be related to *A. radiosa* Maurizio and *A. mucronata* Ziegler, from which it differs in the lack of antheridia.

A. spinosa, which is readily identified by its distinctive gemmae, is much the commoner species. I have found it in several damp, moderately acid soils. *A. stellata* I have found in three situations and in only one of these with any regularity.

I wish to record my gratitude to Mr J. E. Dandy, Keeper of Botany, British Museum (Natural History), for allowing me to examine de Bary's original slides, and to Mr H. V. Newby for advice in the preparation of this manuscript.

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EXPLANATION OF PLATE 12

Fig. 1. *A. spinosa*: habit; note the chain of oogonia and gemmae at top right, clavate and long gemmae bottom right, lateral oogonium with gemma immediately beneath, half way up the left.

Fig. 2. *A. spinosa*: lateral oogonium with apiculus (origin of oogonial branch below the dense gemma at the bottom); note the semi-hypogynous antheridium and the androgynous (monoclinous) one to the right; the stalk has a long appendage.

Fig. 3. *A. spinosa*: intercalary oogonium with androgynous (monoclinous) antheridium.

Figs. 4, 6. *A. stellata*: lateral oogonia and the tip of a sporangium.

Fig. 5. *A. stellata*: habit; note curved oogonial stalks, also the complex oogonium above the sporangium-bearing hypha, and the oogonium and branch central above the large horizontal hypha, recalling Archer's figures of *A. cornuta*.

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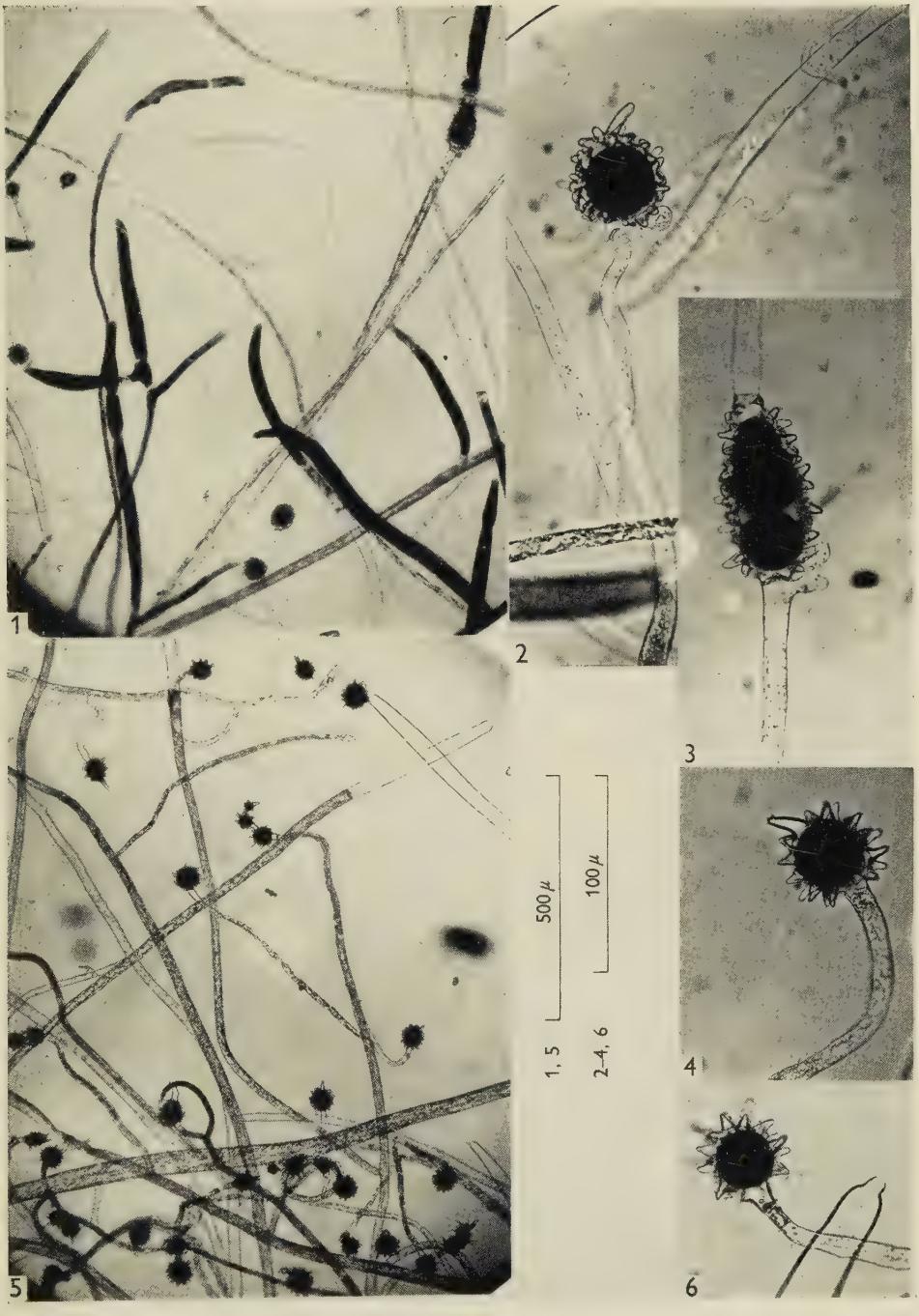

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INVESTIGATIONS ON A DISEASE OF *COFFEA ARABICA* CAUSED BY A FORM OF *COLLETOTRICHUM COFFEANUM* NOACK

I. SOME FACTORS AFFECTING INFECTION BY THE PATHOGEN

By F. J. NUTMAN and F. M. ROBERTS

Coffee Berry Disease Research Unit, Department of Agriculture, Kenya

(With Plates 13 and 14 and 4 Text-figures)

The berry disease of *Coffea arabica*, caused by a form of *Colletotrichum coffeanum* Noack, may have arisen by mutation of the fungus from a mildly parasitic to a virulent form at some time prior to 1922. Conidia from different sources were found to vary in viability and infectivity, those from lesions on ripe berries being superior to those from green, and conidia from pure cultures on potato-dextrose agar being the least satisfactory. Different stages of the crop varied markedly in susceptibility, flowers, very young fruit, and ripe berries being the most susceptible; germination rates of conidia on the surface of flowers were comparable with those on nutrient agar. All parts of the plant can be attacked, and the fungus, which habitually colonizes the maturing bark external to the developing phellogens, can cause a disease of twigs (Elgon die-back) under abnormal conditions.

There is a difference in varietal reaction to attack, berries of the Harar variety and its derivatives being the most susceptible known, and Blue Mountain the most resistant. Wounding increased susceptibility in all varieties; addition to the infection drop of extracts from berries of several different varieties increased susceptibility, with Blue Mountain having the greatest stimulatory effect and Harar none. Varietal reaction of a berry to infection cannot, however, be correlated either with the amount of germination on its surface, or with stomatal numbers, but increased germination does increase the invasion rate in individual flowers or berries. Variation between varieties in susceptibility is ascribed to some chemical or physical difference in the cuticle, making penetration relatively more difficult in the resistant variety. At the optimum temperature of around 22° C., and at a suitable humidity, infection can take place in as little as 2 hr.

The fungus *Colletotrichum coffeanum* Noack (*Glomerella cingulata* (Stonem.) Spauld. & Schrenk) is responsible for a very serious disease of *Coffea arabica* in Kenya Colony. It was first described by Macdonald (1926) who transmitted the disease to wounded berries in the field, and who was later reported by Rayner (1952) to have infected unwounded berries of the variety Harar by spraying detached branches in the laboratory with a suspension of conidia of the fungus. He did not, however, succeed in infecting flowers.

C. coffeanum apparently occurs on *C. arabica* in all parts of the world. Small (1926) reviewed its occurrences in Uganda, where it causes a 'brown blight' of coffee leaves and berries, and Butler (1918) reported it as causing an anthracnose of leaves in South India, and also, but infrequently,

spots on the ripe fruit; he also mentions it as a cause of twig infections in Brazil. It is clear, however, that the causal agent of coffee-berry disease is not the fungus described by Small and Butler and not the same as a mildly parasitic *Colletotrichum coffeanum* found by Macdonald in Kenya in districts where the berry disease was absent. Since, however, the pathogen is morphologically indistinguishable from the non-pathogen, it can be considered as an actively parasitic strain of the widely distributed, but comparatively innocuous, *C. coffeanum* Noack. The perfect stage of the pathogen (*Glomerella cingulata*) has not been found in Kenya, either in the field or in pure culture in the laboratory.

The disease in Kenya is comparatively recent. Macdonald, in 1926, published the first description of a 'Coffee-berry disease' which, from 1922, had been causing up to 75 % losses of crop in two small areas west of the Rift Valley. Since then it has spread considerably in the Colony, breaking out in the Mount Kenya district in 1939; in 1951 it appeared in the main coffee-growing district of Kenya east of the Rift Valley. It has also been reported in the Belgian Congo, and more recently there have been reports of the presence of a coffee-berry disease in other parts of tropical Africa.

Macdonald described a 'brown blight' of ripe berries in districts where the coffee-berry disease was not, at that time, present and considered it to be due to a strain of *C. coffeanum* different from that causing lesions on green berries. He was able to secure a high percentage of successful inoculations on green berries with conidia derived from green-berry lesions, but very few with conidia derived from ripe berries. It seems that the fungus which Macdonald found on ripe berries may well have been the same, or a similar strain, as that referred to by Small and by Butler.

At the present time the situation in Kenya is not that encountered by Macdonald. All stages of the crop are attacked, from the flowers to the fully ripe berries, and an anthracnose (rarely of importance under Kenya conditions) is produced on leaves: we have also found that a die-back of the young green twigs can be caused under rather special conditions, and colonization of the semi-moribund cortical tissues exterior to the developing phellogens occurs. The importance of the latter will be discussed in a later communication. The fungus responsible for all the above-mentioned forms of attack is the same, and conidia from any lesion on any part of the plant will produce typical symptoms on any other. The strain which could attack ripe berries only has now apparently disappeared and conidia from ripe berries are, on the contrary, invariably more viable and more infective than those from green ones.

There does occur, however, a completely non-parasitic strain of *C. coffeanum*, which colonizes the cortical tissues external to the developing phellogens before the formation of true bark, and occurs in districts where coffee-berry disease is absent. This strain, morphologically identical with the pathogen, has never been induced by us to invade even wounded berries of the most susceptible varieties under optimum conditions.

A possible explanation of these contradictory results is that *C. coffeanum* may originally have been present in two strains, one saprophytic and one mildly parasitic, the latter causing the comparatively harmless brown blight

of ripe fruit and anthracnose of leaves, but being unable to attack the more resistant unripe fruit. At some time prior to 1922 the pathogenic strain arose, possibly as a mutant, in West Kenya, and its necessarily more efficient parasitism may well have resulted in the suppression of the weak parasite: in support of this view the saprophytic form has obviously been displaced in the districts where coffee-berry disease is now endemic and cortical tissues in these districts are invariably colonized by the pathogen. There is some indication that the two strains co-exist in the border districts between the diseased and the healthy areas.

Rayner (1948, 1952) suggested that the chemical constitution of the green berries in the districts affected by coffee-berry disease was such that a mildly parasitic *Colletotrichum* capable of causing a 'latent infection' only, could be changed, by growing in such a berry, to the active pathogen. There are many objections to such a hypothesis.

From 1951 onwards losses from coffee-berry disease caused increasing alarm, and intensive investigations were started towards the end of 1955. Bock (1956) published an account of preliminary laboratory work and demonstrated the value of studying the disease on detached berries under laboratory conditions—a technique used by Small, but not, apparently, by Macdonald or Rayner.

This paper reports field observations supplementary or additional to those of previous workers, together with experiments consequent upon these. It also reports studies of some factors affecting spore germination and infection of *Coffea arabica* tissues by the pathogen.

Experimental techniques

Infection experiments were usually carried out on detached berries. These, selected in the field for uniformity, were placed on damp cellulose wadding in covered plastic boxes. Where the work involved the treatment of batches of berries, as, for example, if fungicide treatment was necessary at certain intervals after inoculation, they were placed in clips attached to perforated plastic trays which fitted the bottoms of the boxes. Inoculation almost always consisted in placing a small infection drop of a conidial suspension (*c.* 10,000 spores/ml.) on each berry. The criterion of successful infection was, in all cases, the production of a sporing lesion (Pl. 14, fig. 12). Trouble with casual infections was rare.

Conidia harvested from monospore cultures on various media give erratic infection rates and accurate work is difficult. Germination of such conidia is, in general, poor, and we agree with Bock (1956) in thinking that this might be the reason for the difficulties experienced by earlier workers in infecting unwounded berries with similar inoculum. It is possible, however, to secure a high percentage of infections with such inoculum provided that conidia from freshly isolated cultures are used, and that the spore concentration is adequate (Table 1).

Tests in van Tieghem cells showed that conidia from green berries gave much higher germination rates than those from artificial cultures, that those from ripe berries gave an even higher rate, and that those from young lesions gave a higher germination rate than from old. Freshly extruded

spores from ripe berries develop appressoria after the formation of very short germ tubes, and sometimes germinate directly into appressoria (Pl. 14, fig. 13). Those from green berries tend to have longer germ tubes, often without appressoria. In the work reported here, unless otherwise stated, only spores harvested from fresh lesions on ripe berries were used.

Table 1. *Number of lesioned berries resulting from inoculation with conidial suspensions from various sources*

Source of conidia	Numbers of infected berries. Days after inoculation			Percentage infected
	6	11	17	
Monospore culture on potato-dextrose agar (1)	34/45	43/45	43/45	96
Monospore culture on potato-dextrose agar (2)	30/45	44/45	44/45	98
Culture on green-berry medium	39/45	42/45	45/45	100
Berries infected in field (var. Fr. Miss)	30/35	35/35	35/35	100
3rd transfer monospore culture (p.d.a.)	27/35	31/35	32/35	90
Berries infected in field (var. Harar)	28/35	34/35	35/35	100

Germination rates were studied *in vitro* by the usual techniques, in hanging drops in van Tieghem cells, and on the surface of microscope slides coated with agar. Observation of germination on plant surfaces, essential to a proper understanding of infection processes, was made possible by a stripping technique. An infection droplet of about 10,000 conidia/ml. was placed on the berry or flower surface, between two small spots of indian ink. After an appropriate incubation period the droplet was rapidly dried under a fan, and a thin coating of a colourless (cosmetic) nail varnish was applied. When dry, this was stripped off and mounted in a dilute solution of cotton-blue in lacto-phenol. Very clear casts of the plant surface can be obtained in this way, showing the germinating spores with appressoria, and counts of stomata can readily be made.

Attack on flowers and very young fruit

Attack on the flowers of *Coffea arabica* by *C. coffeanum* has not been previously observed. The flowers are, however, extremely susceptible and, when infection is severe, almost total absence of crop can result. This often escapes notice owing to the ephemeral nature of the flowers. Infection can take place at any stage from that of the pale green, unopened spike, to that of the mature flower. An indication of the losses encountered can be seen in the trees where nodes, having already flowered, are either bare or have a few berries only, often of widely different ages, in place of the usual cluster of sixteen or more. Other causes, can, of course, produce the same effect, but these are usually easily identifiable at the time the losses are being incurred.

The first symptom of attack on the flower is usually a dark brown blotch or streak on the white tissue, and the course of penetration, invasion, and destruction is rapid; this is illustrated in Pl. 13, figs. 2 and 3, which shows attack over a 48 hr. period. Cross-inoculation experiments have confirmed

that the fungus attacking the flowers is identical with that attacking the berries.

Almost as susceptible as the flowers are the very young fruit known as 'pin-heads' (Pl. 13, fig. 6). This expression denotes the club-shaped swollen ovary after petal fall, before it develops the typical berry form. Fruit infected at this stage usually abscisses, and later the only evidence that attack has taken place is the absence of berries at the nodes. When the semi-mature or mature fruits are infected, however, they generally become mummified and the blackened berries often remain adherent for a long time (Pl. 13, fig. 7).

The loss of flowers and young crop has a secondary and not unimportant effect. Generally, over a considerable part of the coffee districts, a main flowering takes place around April. There is a subsidiary and smaller flowering, often suppressed, in October–November. When, as is often the case, a high proportion of the April flowering is lost to disease, the inevitable response is an exceptionally heavy flowering, or series of flowerings, in October–November. This results in there being crop on the trees throughout the year and, in addition to the increased difficulty of plantation management, this continuous bearing may have a not inconsiderable effect on the general inoculum-level of the plantation, and consequently on control of the disease.

The following inoculation experiments were carried out. Twenty flowering branches taken from an infected plantation were enclosed in groups of four in large polythene tubes which were tied so as to leave the lower ends of the stems free. Those in three of the bags were sprayed with a suspension of conidia in water, and in two were sprayed with water as a control. All were placed in deep buckets with a few inches of water; the tops of the tubes were closed for the first 24 hr. after inoculation, after which they were left open for the duration of the experiment. After 7 days, individual flowers and buds were examined and counted; 89 % of those inoculated were found to be infected, but only 13 % of the controls.

Table 2. *Effect of spraying detached flowering branches of* Coffea arabica *with a conidial suspension of* Colletotrichum coffeanum

Four-branch group no.	Inoculated		Group no.	Control	
	Healthy	Infected		Healthy	Infected
I	19	206	VI	206	11
II	33	175	VII	131	23
III	27	86	VIII	61	11
IV	5	102	IX	124	8
V	6	94	X	109	27
Totals	90	663		631	80
Percentage diseased	88			11	

In a second experiment, using forty branches cut from the same plantation, 3 days only were allowed for incubation after inoculation, but twenty of the inoculated branches, and ten of the controls were left for a further 6 days in humid conditions to determine the amount of infection which

might develop in the pin-head berries. The results, shown in Table 2 below, were that 88 % of the flowers on the inoculated branches became diseased, as against 11 % on the controls. In the batch which was incubated for the longer period, 71 % of the pin-heads on the inoculated branches were diseased, and 19 % on the controls, most of the latter bearing small discrete lesions of the fungus, whereas those on the inoculated branches were completely decayed, indicating multiple infections.

The following infection experiment was carried out in the field. In the plantation from which material for the above experiments was obtained, two comparable branches were selected on each of nine trees a few days after flowering. Most of the crop was then in the pin-head to young berry stage. One branch on each tree was enclosed in a polythene bag, sprayed with water, and the top of the bag left open: the second branch was treated similarly but was sprayed with a spore suspension. This was done at sunset, in dry warm conditions. After 7 days the eighteen branches were brought to the laboratory and kept with their cut ends in water for 4 days before assessing the results, in which only pin-heads and very young berries were included, and Table 3 shows that 33 % of the inoculated young crop was infected and 10 % of the controls.

Table 3. *Results (as number of berries infected) of spraying branches bearing young crop in the field with conidial suspension of* Colletotrichum coffeanum

Tree no.	Inoculated		Control	
	Healthy	Infected	Healthy	Infected
1	52	18	93	21
2	143	47	66	12
3	46	14	44	0
4	200	17	73	3
5	18	94	78	6
6	38	12	112	6
7	72	43	213	30
8	28	15	70	9
9	87	34	85	8
Totals	684	294	834	95
Percentage infected berries	33		10	

The whole of this series of experiments was carried out during a prolonged period of warm, dry weather and the fungus was not, therefore, very active in the field. Even in these conditions, however, and in a plantation where the level of disease incidence was not at that time very high, 11–13 % of the flowers, and 10–19 % of the young fruit, were lost from natural infection when placed in conditions of temperature and humidity comparable with those in a plantation during the hours of darkness. Under favourable conditions, copious production of conidia from infected flowers can be observed in the field, and it is evident that much of the observed loss of pin-heads following attack on the flowers is a result of the concentration of inoculum thus built up. Pl. 14, figs. 8 and 9, illustrates losses in the pin-head stage resulting from inoculation of the stipular bract. After 24 days,

both leaves and one peduncle have abscissed, the fungus in the stipular bract is sporing freely and invasion of the cortical tissues of the stem from the bract is obvious.

Elgon die-back

The dying-back of the tips of branches of the coffee tree is a common phenomenon, and often occurs as a result of over-bearing or of drought. The cause of a condition distinct from these, however, and known in Kenya as 'Elgon die-back', has been observed; it was first associated with coffee in the Mount Elgon district, but was later found to occur in all parts of the Colony. Thorold (1945), reporting the results of a lengthy investigation, concluded that the fungi isolated from affected material, mainly species of *Phoma* and *Colletotrichum*, were secondary, and stated that inoculation of trees with these failed to reproduce the disease.

The expression 'Elgon die-back', as the writers understand it today, refers to the death of individual branch-ends on otherwise healthy trees. The first field symptom is often that a pair of young leaves blacken from the stalks and up the mid-ribs, or one or more dead or dying leaves may indicate the starting-point of a wilting of the stem above. More usually, leaves die successively up the stem towards the tip, sometimes at a more rapid rate on one side than on the other, until the growing point blackens and dies, with the final appearance indistinguishable from a tip die-back. Species of *Phoma* and *Fusarium* sometimes, and *Colletotrichum* always, have been isolated from affected branches.

Contrary to the findings of Thorold, we have found that the condition can readily be reproduced in the laboratory by inoculation of twigs of *C. arabica* with *C. coffeanum* derived from berries infected with coffee-berry disease. Possibly Thorold only attempted inoculation in the field. Of many similar experiments, all giving comparable results, the following is typical. After rinsing in water and removing any rusted or insect-damaged leaves, thirty-six branches of *C. arabica* var. Harar were divided into three groups and the younger green internodes, in which phellogen had not yet been differentiated, were treated as follows. With a camel-hair brush twelve of the branches were brushed lightly over the stems and stipular bracts with a water suspension of conidia harvested from infected berries; twelve were lightly pricked with a needle before applying the suspension; the remaining twelve were brushed with sterile water. The branches were placed in large containers with their cut ends in water, and kept in the dark at a high humidity for 24 hr.; they were then maintained in the light.

After 9 days, many of the stipular bracts on the treated branches had darkened, and one terminal leaf had died. After a further 15 days, there was little difference between the wounded and unwounded treatments in the amount and kind of symptoms, although sporing lesions often formed over the site of a puncture; the effect is somewhat similar to that resulting from hail damage in the field (Pl. 14, figs. 10, 11). Of the twelve branches which had been inoculated without wounding, two were attacked in the stipular bracts only, eight were dead (the tops having been cut off by the fungus at some intermediate point, one showed blackening running up the

mid-ribs at the leaf bases only, and one had lesions spreading from the stipular bracts and bearing acervuli of the fungus. It was noticed that, in the great majority of twigs which developed symptoms, the tips were the last part to become infected. All the above symptoms have been observed in the field. At the conclusion of the experiment, all the control branches were in a fresh condition and exhibited no sign of infection or debility.

Elgon die-back is not a serious affection of coffee trees except occasionally in certain districts; its prevalence in the Mount Elgon district may be correlated with frequency of hail there, for although *C. coffeanum* can, under appropriate conditions, penetrate the epidermis of the stem, entry is facilitated by wounds. The fungus normally colonizes the cortex, growing externally to the developing phellogens, but does not penetrate these. In twigs where phellogen has not yet formed, however, or which have been mechanically damaged, it can penetrate to the cambium and cut off the tissues above the point of penetration, as can be seen in sections of stems just above the junction between diseased and healthy tissue. Death of very young leaves, similar in appearance to that caused by the berry-disease fungus, can also be caused by a species of *Phyllosticta*, but this has been observed to cause the death of the extreme tips of branches only. Many specimens of Elgon die-back, including collections from the Mount Elgon districts, have been examined without success for the perfect stage of the fungus.

Varietal reactions to *Colletotrichum coffeanum*

Previous workers have observed that certain varieties of *C. arabica* differ widely in their reaction to *C. coffeanum*. For example, the varieties Harar and S.L. 30 are highly susceptible and are severely attacked in the field under conditions in which other varieties escape infection. Blue Mountain, on the other hand, has considerable field resistance, and will yield a crop in circumstances where total loss from other varieties is experienced. This varietal effect shows up clearly under laboratory conditions, a large number of trials with Harar and Blue Mountain averaging 96·0 % successful inoculations with the former, compared with 21·3 % for the latter. Bock (1956) has published data indicating that the environment under which the fruit has developed may also affect reaction to the fungus: it is unfortunate that only two plants of one clone were available to this worker—one in each environment.

The cause of this variation is manifestly of importance. Resistance of a berry to attack can only be due to: (*a*) some internal factor which makes the living cells of the berry less easily invaded by the fungus; (*b*) some condition on the berry surface which affects germination in a differential manner; (*c*) some chemical or physical variation in the cuticle affecting ease of penetration by the infection peg, and, of course, any combination of these.

The first possibility was studied by comparing the number of infections which result from placing drops of conidial suspensions directly on berry surfaces, with those produced on berries from which thin discs of epidermis had been removed by tangential cuts with a sharp razor. Table 4 shows that the effect of wounding made the two more resistant varieties, Blue Mountain and Kents, as susceptible as Harar.

A further series of experiments was carried out on the effect of a slight prick with a needle through the infection drop. Table 5 summarizes the results, and provides further evidence that there is little difference between varieties in the ease with which the pathogen can develop in the internal tissues, since all, when wounded, are equally susceptible; variation in reaction of varieties must be sought, therefore, in phenomena associated with penetration of the cuticle.

Table 4. *Number of berries infected (out of forty) in three varieties, with and without wounding*

Days after inoc.	Harar		Kents		Blue Mountain	
	Unwounded	Wounded	Unwounded	Wounded	Unwounded	Wounded
5	1	33	0	24	0	26
9	22	34	4	36	1	31
12	32	36	5	38	1	33
16	39	37	8	38	4	36
Percentage infected	97·5	92·5	22·5	95·0	10·0	90·0

Table 5. *The effect of slight wounding on invasion of berries of three varieties of* Coffea arabica *by* Colletotrichum coffeanum

(Number of berries in each trial given in parentheses.)

Variety	Percentage infection	
	Pricked	Unpricked
Harar	85 (200)	90 (200)
Blue Mountain	78 (200)	22 (200)
Bourbon	88 (50)	4 (150)

Table 6. *Effect of conidia concentration on lesion production after* 6 *days in three varieties of* C. arabica

	No. berries infected (out of 90). No. conidia/ml.					
	10^6	10^5	10^4	10^3	10^2	10
Harar	87	36	5	1	2	0
Blue Mountain	22	11	13	9	2	6
S.L. 30	86	50	21	7	5	1

The effect of wounding is twofold; it permits direct access of the fungus to the internal tissues, and it allows direct leaching of nutrients into the infection drop. All workers have commented on the low and irregular germination of the conidia of *C. coffeanum* and the high concentrations necessary to secure infections have been studied by Bock (1956) and also by the writers. Consequently, any large increase or decrease in germination rate, from whatever cause, would be expected to increase or decrease lesion production with any inoculum containing less than about 10^4 spores/ml. Table 6 illustrates the effect of spore concentration on lesion production.

Various nutrients in high dilutions, e.g. dextrose and mannitol, markedly increase germination rate, as do dilute extracts of coffee berries. In one experiment, thin peelings of the skin of berries of the Harar variety were

allowed to diffuse into distilled water until the solution became faintly coloured. Drops of this were put into Van Tieghem cells at full, 1/2, 1/4 and 1/8 dilutions, and were seeded with conidia of *C. coffeanum* from a monospore culture on potato-dextrose agar. After 18 hr. the control and the undiluted infusion showed no germination; germination in the 1/2, 1/4, and 1/8 infusions was 6, 23 and 27 % respectively.

The following experiments were made to see whether any substance was present in berries of different varieties which had a differential effect on invasion by the fungus. The first experiment was with berries of the varieties, Harar, Blue Mountain and Kents. Four containers of each variety, each with thirty-five berries, were inoculated with conidia harvested from field-infected berries. After inoculation, a fragment of berry rind, approximately 2 mm. square, was added to all of the infection drops. Thus, for each variety, the first treatment was the addition of a fragment of Blue Mountain tissue; the second, of Harar tissue; the third, of Kents tissue; while the fourth series received no tissue. The addition of tissues of any variety increased lesion production, the total of berries developing lesions in all varieties with no tissue added being 35/105; the addition of Harar, Blue Mountain, and Kents tissues gave 56, 66 and 67/105 infected berries respectively.

In a second experiment, in which Bourbon was used in addition to the above varieties, infusions of berry skins were mixed in equal proportions with the infection drops. Table 7 illustrates the results, which again show that extracts of Blue Mountain exhibited a marked stimulatory effect, while extracts of Harar had, if anything, a depressing effect on infectivity. There is, thus, no evidence of any fungistatic component in tissue extracts from the resistant variety Blue Mountain; on the contrary, as can be seen in the summary of results at the foot of the table, its extracts show a greater stimulatory effect than do those of other varieties, including the extremely susceptible Harar.

With the object of ascertaining whether infection was differentially affected by substances diffusing through the uninjured cuticle during the infection process, lesion production on different varieties was compared using two types of inoculum. The first was a simple suspension of conidia in water. The second, of the same concentration, was a suspension of conidia in water in which berries of the different varieties had been steeped for the previous 24 hr. These two types of inoculum are compared with another in which extracts of the skins of berries were mixed, as before, with conidial suspensions, bringing the spore concentrations in all cases to the same level. Text-fig. 1 illustrates the results, and contrasts the increase in infectivity when extracts from cut skins are used with the slight reduction when diffusion products are used.

Experiments were also made in which berries of Blue Mountain and of Harar were placed in pairs in contact, with the infection drop common to both; thus, any effect of one berry on spore germination would necessarily affect lesion production in both equally. The results of these tests were invariably negative, the number of lesions developing in each variety being as would have been expected had they been inoculated separately.

Finally, actual germination on the surface of twelve berries each of

Harar, S.L. 30, and Blue Mountain was studied. The berries were inoculated with infection drops containing 12,000 conidia/ml. After 24 hr. the drops were dried under a fan, and the spores stripped from the surface for examination. Appressoria only were counted as effectively germinated conidia, 400 spores being counted on each slide, giving a total of 4800 counts for each variety. The results are shown in Table 8.

Table 7. *Effect of extracts from the berries of four varieties of* Coffea arabica *on reaction to infection by* Colletotrichum coffeanum

		No. infected berries (out of 35). Days after inoculation					Total infected
		6	8	12	16	20	175
Kents	With no extract	0	2	3	8	14	—
	With Harar extract	0	1	2	9	10	—
	With Blue Mt. extract	1	1	9	19	23	—
	With Bourbon extract	0	1	4	10	14	—
	With Kents extract	0	1	9	13	16	77
Harar	With no extract	2	16	31	35	35	—
	With Harar extract	2	22	35	35	35	—
	With Blue Mt. extract	5	19	34	35	35	
	With Bourbon extract	5	17	34	35	35	—
	With Kents extract	0	27	31	35	35	175
Blue Mt.	With no extract	0	2	9	11	14	—
	With Harar extract	1	5	14	14	17	—
	With Blue Mt. extract	0	4	13	20	25	—
	With Bourbon extract	0	4	8	16	21	—
	With Kents extract	0	4	14	17	21	98
Bourbon	With no extract	0	2	13	15	18	—
	With Harar extract	1	2	12	14	17	—
	With Blue Mt. extract	1	1	13	26	29	—
	With Bourbon extract	0	4	13	14	15	—
	With Kents extract	0	2	6	7	9	88

Summary of results (all varieties)

	Total infected berries (out of 140)
With no extract	81
With Harar extract	77
With Blue Mt. extract	112
With Bourbon extract	85
With Kents extract	81

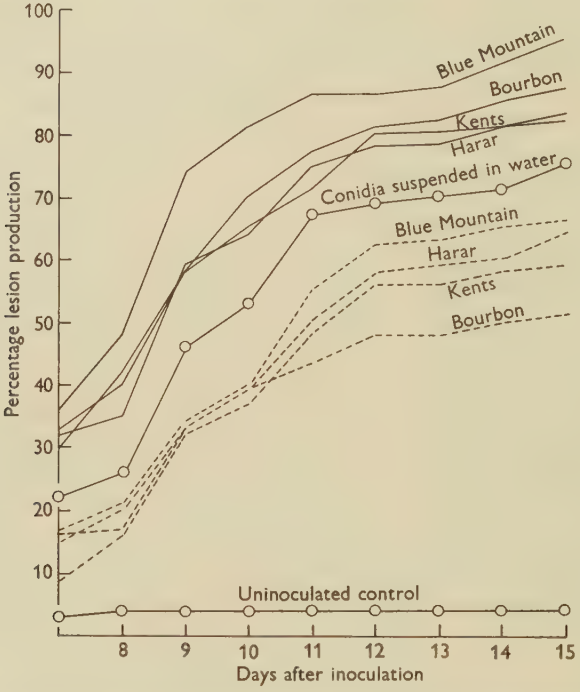
Table 8. *Numbers of appressoria and stomata on berries of three varieties, and numbers of infected berries*

	Blue Mountain	S.L. 30	Harar
Appressoria (out of 4800 conidia)	772 (16%)	1339 (29%)	655 (14%)
Stomata	345	548	291
Infected berries (out of 90)	22	86	87

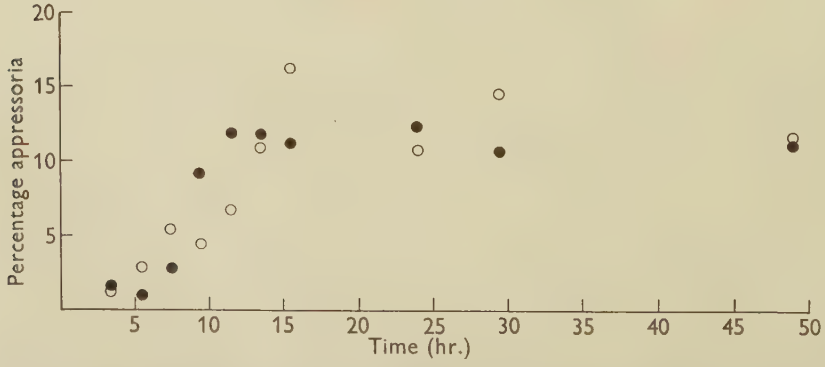
It is clear that there is no significant difference between germination rates on the surface of Blue Mountain and of Harar berries, one resistant to the disease and the other very susceptible. S.L. 30, which is also very susceptible to coffee-berry disease, did give a higher germination rate, but for

all three there is no apparent correlation in varietal reaction between appressoria formation, stomatal numbers, and lesion formation.

Text-fig. 2 illustrates the rate of formation of appressoria on the berry surfaces of the two varieties, Blue Mountain and Harar, at 22° C., each point being the mean of counts of 400 spores on each of five berries, making a total of 2000 counts. There is no significant difference between either the



Text-fig. 1. Entire lines: extracts of berry skins added to conidial suspensions. Broken lines: conidia suspended in water in which uninjured berries had been steeped for 24 hr.



Text-fig. 2. Formation of appressoria of *C. coffeanum* on surface of berries of Blue Mountain (black) and Harar (white) at 22° C. Each point represents the mean of counts of 400 spores on each of five berries, i.e. of 2000 spores.

rate of formation, or the final percentage, of appressoria formed on these two varieties, although one is the most resistant known, and the other the most susceptible.

To summarize, therefore, it is clear that there is no differential reaction to the fungus by the tissues of berries of the different varieties of *C. arabica*; while differential germination rates do affect lesion production on any given variety, differential germination rates on berries of different varieties are not responsible for varying varietal resistance; there is no fungistatic component in the tissues of the resistant varieties; the sole cause of the difference in susceptibility between varieties lies in the ease, or otherwise, with which the infection peg can penetrate the cuticle. This may explain the results of Bock (1956), referred to previously; the extreme difference in the environment of the two bushes from which his material was derived may well have affected cuticular development. Under normal field conditions, however, variations in reaction correlated with environmental conditions have not been observed.

Relative susceptibility of flowers and berries, and variation in susceptibility between berries of the same variety

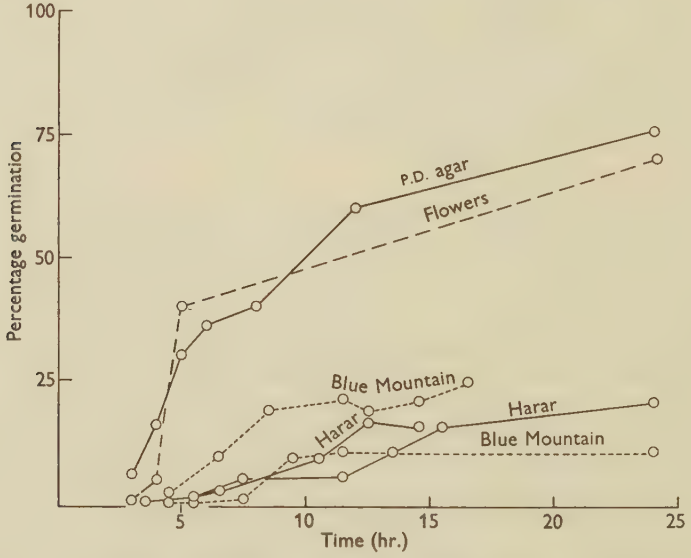
There is considerable variation in reaction to the pathogen by the crop at different stages of maturity, and also by individuals at the same stage. Flowers, very young berries, and fully ripe fruit are extremely susceptible to attack; the pre-ripening stage, however, is a lengthy period during which the endosperm matures and develops without appreciable change in the size of the green berry, and during this stage a phase of semi-resistance to the fungus occurs. There is also considerable variation in resistance between individual berries of the same batch, even when these are of the same variety and are carefully selected for apparent uniformity. The following experiments were carried out in an attempt to elucidate these observations.

Table 9. *Germination of conidia of* Colletotrichum coffeanum *on flowers and on berries of* Coffea arabica

Flower (or berry) no.	No. germinated conidia											
	Flowers					Total	Berries					Total
1	44	49	30	38	36	197	1	2	0	0	0	3
2	63	91	82	66	47	349	6	5	5	5	2	23
3	28	26	24	24	26	128	15	18	11	8	9	61
4	38	22	15	29	45	149	1	3	1	3	1	9
5	39	36	19	26	27	147	3	3	3	4	1	14
6	60	43	42	49	47	241	1	0	3	1	0	5
Totals						1211						115
Percentage infection						40·3						3·8

Using the stripping technique, germination on flower buds immediately prior to opening was compared with that on berries. The incubation period was 18 hr., and 500 conidia were counted on each of six flowers and berries. As can be seen in Table 9, the amount of germination on the flowers was more than ten times that on the berries.

Text-fig. 3 illustrates germination rates on slides coated with potato-dextrose agar, on flowers, and, in two separate experiments, on berries of the highly susceptible variety, Harar, and of the resistant Blue Mountain variety. These curves, being derived from experiments made with different batches of conidia, are not so directly comparable as are the figures in Table 9. They do, however, show that germination on flowers is similar to that on nutrient agar, and probably maximal, while that on berry surfaces is much lower. Part, at least, of the extreme susceptibility of flowers is probably the result of the greatly increased germination rate on such surfaces.



Text-fig. 3. Germination of spores of *C. coffeanum* on agar compared with that on flowers and on berries of two varieties of *C. arabica*.

It has previously been shown that, although the main cause of varietal variation in reaction to *C. coffeanum* is cuticular, increase in germination rate on the berry surfaces increases the invasion rate, and the following experiments were carried out to determine whether differential germination could be responsible for part, at least, of the variation in susceptibility with age, and from berry to berry. The results of these experiments are summarized in Table 10.

Berries of different ages, selected for uniformity of appearance in each group, were inoculated and incubated; germination was then measured by the stripping method. Ten berries per treatment were usually used, and 500 conidia counted on each. One treatment was a light needle-prick through the infection droplet. This was done because it was thought possible that the berry to berry variation might be a result of invisible insect-punctures or abrasions. Appreciable variation in germination on pricked berries would, however, discount this supposition. This proved to be the case; on the five berries of the young pricked series the counts of appres-

soria per individual berry, out of 500 conidia, were 130, 88, 21, 69 and 26, and it is clear that this is enough to account for much, if not all, of the variation in attack. An extreme example of variation between apparently similar berries occurred in one series of ten unwounded berries of 'French Mission' coffee which is genetically heterogenous; the numbers of appressoria per 500 conidia were 12, 16, 234, 3, 11, 1, 4, 17, 4, and 27 on individual berries. Reference to Table 9 (berry no. 3) shows the same phenomenon; in flower no. 2, a similar variation occurs on flower surfaces. The cause of this variation is unknown.

Table 10. *Percentage germination of conidia of* Colletotrichum coffeanum *on the surface of berries of different varieties, ages, and treatments*

Exp.	Variety	Spore source	No. conidia counted	Age of berry and treatment				
				Y	Y/P	M	M/P	YY
I	Guatemala	Green berries	2500	8·1	13·2	5·2	6·2	—
II	Guatemala	Red berries	5500	—	—	1·5	8·6	—
III	Kents	Red plus green	5000	—	—	3·9	4·5	—
	Blue Mountain	Red plus green	5000	—	—	3·5	5·5	—
	Harar	Red plus green	5000	—	—	1·4	3·4	—
	Fr. Mission	Red plus green	5000	9·0	11·1	4·6	4·0	—
	Fr. Mission	Red plus green	5000	—	—	1·9	—	12·7

Incubation periods: 16 or 24 hr.

M, mature berries, fully developed, hard, green; Y, approx. half the size of the above, with no hardening of endosperm; YY, very immature, less than 0·5 cm. diameter, not yet fully rounded; P, berries pricked by needle through infection droplet.

Note. All the differences between percentage germinations in each row are statistically significant.

It would thus seem, from the summarized results shown in Table 10, and the above discussion that:

(i) Germination on the surface of mature berries is much less than that on young ones, which in turn is less than on very immature berries; this is in the same order as their known resistance to infection.

(ii) Slight wounding markedly increases germination rate, and, as shown previously, also increases the susceptibility of the berry to attack.

(iii) Germination rates on berries of different varieties bear no relation to their susceptibility.

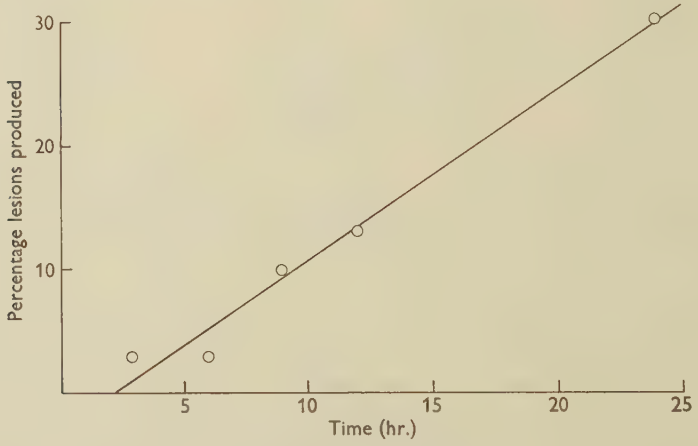
It is possible to conclude, therefore, that some, at least, of the variation in susceptibility between flowers and berries, and of berries with age, is the result of variation in the germination rate of conidia on their surfaces; that a similar variation is responsible for some, if not all, of the berry-to-berry variation in the same batch in any one variety; but that differential germination rates are not responsible for variation in susceptibility between varieties, thus confirming previous experiments.

The time taken for invasion

When germinated spores are stripped from the epidermis of a berry, those appressoria which are fully developed and dark in colour can readily be seen to have formed infection pegs. It can be assumed that by this time

penetration either is being, or has been, effected, and the time taken for this stage to be reached has been used by us as a measure of the threshold period for invasion.

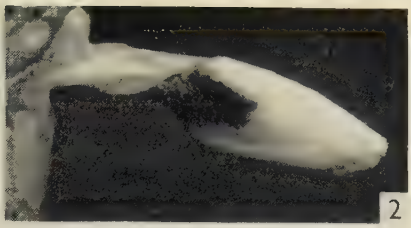
The rate of formation of appressoria on the surface of berries of the susceptible variety Harar and the resistant Blue Mountain at the optimum temperature of 22° C. is shown in Text-fig. 2. Each point on the curve is the mean of a count of 2000 conidia, 400 on each of five berries. It is clear that there is no significant difference in germination of conidia on the surface of berries of Harar and of Blue Mountain, and this is in agreement with previous evidence. The relationship between the formation of appressoria and time is clearly linear for the first 15 hr. or so, formation of appressoria being well advanced after $4\frac{1}{2}$ hr. Many similar experiments agree in placing the first formation of appressoria at about 4 hr. after the conidia are placed on the berry surface, at 22° C.



Text-fig. 4. Relation between length of infection-period and number of lesions produced (for explanation see text).

A more direct method was as follows: batches of berries on trays were inoculated in the usual manner, and after successive intervals lots of 120 berries each were withdrawn and the infection-drops quickly dried in a warm air-current; the berries were then kept dry for 24 hr., a period known to be long enough to kill most ungerminated spores, before being replaced in humidity chambers for the remainder of the experimental period. Text-fig. 4 summarizes the results and shows that lesion formation can take place when the infection period is as short as 3 hr. and that this increases in a linear manner with time for another 20 hr. or more. A similar experiment gave a threshold period of approximately 2 hr.

It is clear from the foregoing that while a very small percentage of spores can cause infection after as short a period of time as 2 hr., the number of lesions formed increases in a linear manner for a period of between 15 and 24 hr. It follows, therefore, that the amount of infection produced in any



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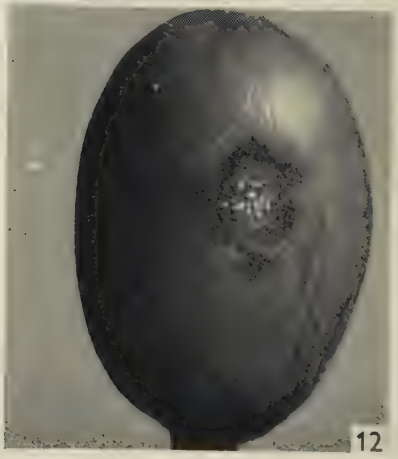
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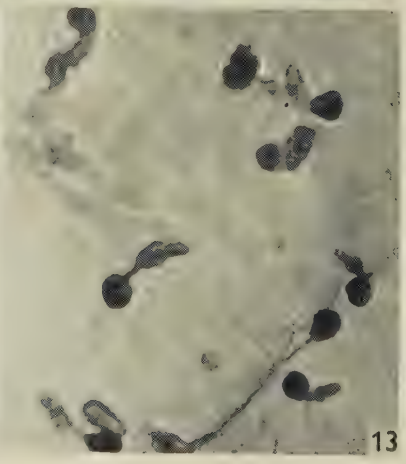
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given set of circumstances is, as might be expected, a function of the length of time that appropriate humidities are maintained on the berry surface, up to about 15 hr.

Distribution of the pathogen

The pathogenic form of *Colletotrichum coffeanum* is at present restricted, more or less sharply, to altitudes of over 6500 ft. The problem of this restricted distribution, when a difference of 200 ft. or less in altitude often represents freedom from, or presence of, the berry disease, is the subject of discussion in a later paper. In this the relations of the fungus with humidity and temperature will be discussed, together with the bearing of these factors on the distribution of the disease.

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EXPLANATION OF PLATES 13 AND 14

Plate 13

Fig. 1. Flowering branch of *C. arabica* showing dark lesions caused by *C. coffeanum* on some of the flowers.
Fig. 2. Naturally infected flower bud. × 5.
Fig. 3. The same, 48 hr. later. The lesion is now producing spores.
Fig. 4. Coffee berry with active lesions. × 3·5.
Fig. 5. Coffee berry with inactive, 'scab' lesions. These are superficial, develop under adverse climatic conditions and eventually change to the active form. Black acervuli are present. × 3·5.
Fig. 6. Very young fruit (pinheads) showing multiple microscopic lesions. × 25.
Fig. 7. Spray of coffee berries: some with lesions, some mummified.

Plate 14

Fig. 8. Healthy node, showing leaf petioles and young crop. × 4.
Fig. 9. The same, 24 days after inoculation of the stipular bract. This is now producing spores; both petioles have been invaded and have abscissed, as has one peduncle.
Fig. 10. Apical bud after inoculation. Two semi-mature leaves have already abscissed, and the remaining very young ones show necrosis advancing up the mid-rib. × 8.
Fig. 11. Inoculated stem, showing production of sporing lesion. × 4.
Fig. 12. Sporing lesion produced on the surface of a berry 6 days after it had been inoculated with a spore suspension.
Fig. 13. Spores, on the surface of a coffee berry, germinating to appressoria. × 650.

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PLEURAGE GWYNNE-VAUGHANIAE SP.NOV.

By WINIFRED M. PAGE
Birkbeck College, University of London

(With 1 Text-figure)

Pleurage gwynne-vaughaniae sp.nov. growing on rabbit dung is described and illustrated.

Pleurage gwynne-vaughaniae sp.nov. (Fig. 1)

Perithecium piriforme, circa 1·0 × 0·5 mm. longum cum paucis, longis, simplicibus pilis in ima parte et numerosis, brevibus, fuscis, fasciculatis pilis in superiore parte. Ascus cum octo distichis sporis. Ascospora e nigra et ovali parte (40–46 μ longa, 22–25 μ lata), et appendice cellulari (20–30 μ longa, 6 μ lata) ex basi dependenti consistens, et appendices gelatinosas ad apicem et ad basim ferens. Appendices gelatinosae apicales quattuor numero, 30–60 μ longae, 3–4 μ latae ad ipsarum basim, $< 1\ \mu$ ad apicem. Appendices gelatinosae ad basim, duae aut tres aut quattuor, ex vagina gelatinosa cellularem appendicem cingente.

Habitat: In fimo cuniculorum, Leonard's Lee, Sussex, Anglia, 11. ii. 58. Typus: Herb. I.M.I. 77666.

For many years I have been interested in the sordariaceous fungi which grow on rabbit pellets. So far most of those encountered have been referable to described species, but recently perithecia of what seems to be an undescribed species of *Pleurage* were encountered in abundance on a sample of rabbit droppings from Leonard's Lee, Sussex. Numerous attempts to obtain it in pure culture have been unsuccessful and the description given here is based on perithecia from the natural substratum.

The dark brownish grey perithecia are rather large (*c.* 1 mm. × 0·5 mm.). The lower, swollen region is semi-transparent and has long simple hairs which vary considerably in number from specimen to specimen but are never very numerous. The neck region is beset with short agglutinated, barely projecting, dark brown hairs giving it a speckled appearance. The ascus contains eight spores in roughly biseriate arrangement. Its apical region is noticeably thickened and this is often discharged with the spores as a thimble-shaped cap clearly visible in a spore-deposit caught on a glass slide. In this respect the fungus resembles *P. curvula* and *P. fimiseda*. It is the ascospores that really characterize the species. They are relatively large and possess a basal straight, cylindrical, cellular appendage. The main part of the spore, the black part, is rounded at the apex and truncated in a characteristic manner at the base where it joins the cellular appendage. The black part is 40–46μ × 22–25μ. From around the apical pole arise four mucilaginous appendages which taper gradually to very fine ends often entangled with one another. In freshly discharged spores these appendages may be drawn out to a considerable extent. The cellular appendage is cylindrical, hyaline and with distinctly granular cytoplasm.

Surrounding it, like a fingerstall on a finger, is a mucilaginous sheath from the free end of which several (usually four) long, tapering strands of mucilage arise. Although the sheath fits the sides of the cellular appendage fairly tightly, especially near its base, it is usually significantly longer, so that there is a distinct gap between the tip of the cellular appendage and the end of the tubular sheath. Sometimes the sheath becomes pulled off

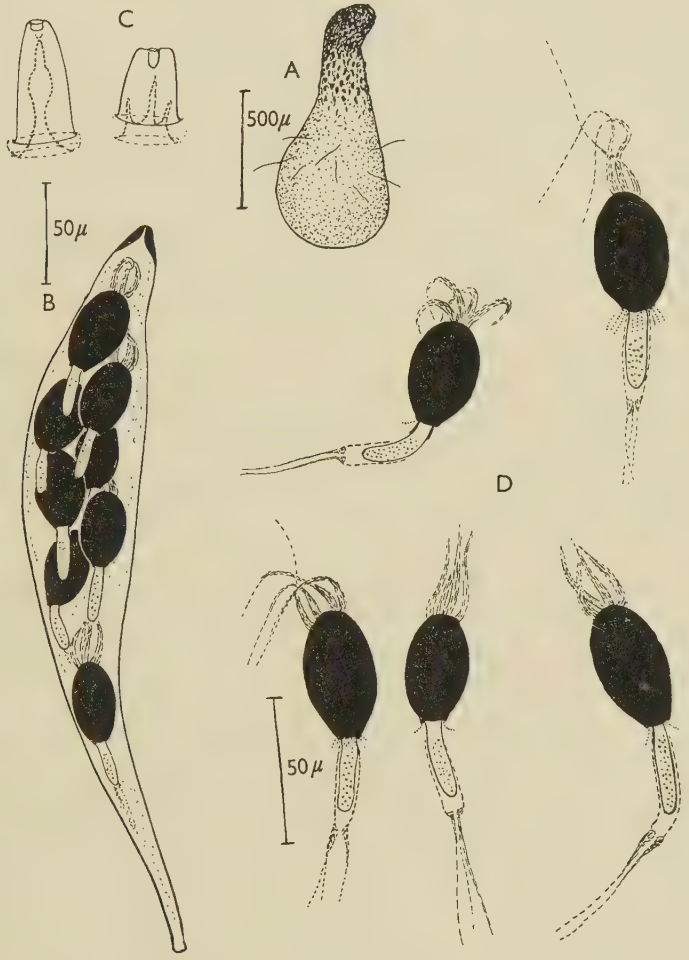

Fig. 1. *Pleurage gwynne-vaughaniae.* A, Perithecium; B, ascus; C, discharged ascus caps; D, spores.

completely and lies quite separate from the spore in a deposit caught on a slide. In addition to the apical and basal mucilaginous appendages, a skirt of very fine short mucilaginous threads can sometimes be seen arising from the base of the black region of the spore. They are very delicate and are visible only in freshly discharged spores and sometimes appear to be

absent even from these. They are much less definite than the mucilaginous processes in a similar position in *P. decipiens* and *P. pleospora*.

The only described species of *Pleurage* with four apical mucilaginous appendages on the spore is *P. vestita*, a common species in North America but not yet reported from this country. The spore of *P. vestita* also has four basal appendages on the free end of the cellular appendage, but these are attached directly and do not arise from a mucilaginous sheath as in the species here described. Further, the shape of the cellular appendage is not quite the same in the two species since in *P. vestita* there is no constriction towards the base nor is a skirt of fine appendages ever present. Finally, the size of the spores, excluding appendages, is significantly greater in *P. gwynne-vaughaniae* than in *P. vestita*.

This new species is named in honour of Prof. Dame Helen Gwynne-Vaughan.

My thanks are due to Prof. C. T. Ingold for making the drawings for Fig. 1.

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METHOD OF UREDOSPORE DISPERSAL OF THE COFFEE LEAF-RUST FUNGUS, *HEMILEIA VASTATRIX*

By F. J. NUTMAN, F. M. ROBERTS and K. R. BOCK
Coffee Berry Disease Research Unit, Department of Agriculture, Kenya

(With Plates 15 and 16)

Experimental evidence is presented against the view that uredospores of the rust, *Hemileia vastatrix*, are normally distributed by wind, although this method of dispersal does apparently occur at a very low level. The uredospores of the rust adhere strongly to each other, to leaves, and to any surfaces however smooth. Their mutual adhesion is broken down by water, and it is concluded that the periodic build-up of rust epidemics is almost entirely a result of spore-dispersal by water-splash. This observation has an important bearing on the problem of control.

Hemileia vastatrix Berk. & Br., the causal agent of leaf-rust in coffee, produces pustules of uredospores on the lower surface of the coffee leaf. The spores are shaped rather like the segments of an orange, and have a very roughened surface. They are borne on hyphae which protrude through the stomata, and thus a young pustule comprises a large number of small clusters: later, the surface becomes encrusted with irregular masses of detached spores, concealing the discrete spore-clusters below (Pl. 15, figs. 1, 5).

During the course of an attempted study of the distribution of *H. vastatrix* in the air, using the trap described by Harrington, Gill & Warr (1959), the spores were found to be excessively rare at all times of the day, under widely differing conditions of wind, rain and sun. Even when the traps were set up inside a heavily affected plantation at a time when picking, which involves considerable mechanical disturbance of the leaves, was in progress, few spores were trapped. The traps were also operated inside a laboratory where many thousands of leaves were being inspected for counts of rust lesions, with the same results. Moreover, data obtained during a series of field trials on control methods proved difficult or impossible to explain if distribution of inoculum is, in the main, effected by wind, and it thus became necessary to investigate whether, in fact, this is so.

Wind velocity inside coffee plantations

Kirkpatrick (1935) made an extensive study of the relation between wind velocity in the open and that inside plantations. This work was carried out at Kiambu, one of the main coffee-growing districts of Kenya, some of his data being obtained from the same plantation in which our spore-trapping was attempted. His results show clearly the mild character of the winds in this district. For example, in one series of 158 recordings, the mean wind-speed in the open was only 4·8 m.p.h., and on only three occasions did it

reach 10 m.p.h. He mentions, however, gusts of several times the average velocity.

Information supplied by the East African Meteorological Department, dating mainly from after the time when Kirkpatrick did his work, confirms his results. These data are from a site, at 33 ft. above ground level, some 10 miles from Kiambu and about 350 ft. higher. Records over the past 24 years show that only 81 gusts (of about a minute's duration) of 40 m.p.h. and higher have been recorded, and of these 25 occurred in 1933. On two occasions, 3 years elapsed without a single recorded gust, while only three occurred between 23 April 1942 and 5 February 1948. The average annual frequency is 3·4 gusts, with an average velocity of 42 m.p.h. and an absolute maximum of 54 m.p.h. (on two occasions), corresponding to velocities of 33 and 42 m.p.h. respectively at 6·5 ft. above the ground.

Kirkpatrick's results indicate that the proportion of wind penetrating a plantation increases with the external wind velocity to a maximum, after which it remains constant. Thus, with the strongest winds recorded by him (7·5 m.p.h. outside the plantation), only 7·0 % penetrated between the trees at 8 ft. spacing, while inside the canopy the air-speed was naturally less. The average rate of air movement inside such a plantation is unlikely, therefore, to exceed 0·5 m.p.h. In plantings at wider spacing (10 ft.), 45 % of the wind can penetrate and average speeds of 2 m.p.h. and maximum speeds of 4·5 m.p.h. are experienced between rows.

It is difficult to estimate the influence of gusts, since these are about three times the velocity of the strongest wind recorded by Kirkpatrick. It seems probable, however, that the wind speeds produced by them in the plantation would not be greater than 2·5 m.p.h. at an 8 ft. spacing, and 14 m.p.h. at a 10 ft. spacing; they may well be much less and as they occur for an average of only about 3 min. a year, they are not considered to be of much importance.

Experiments

Effect of air-currents on pustules

(1) Leaves bearing heavily sporing lesions were brought to the laboratory with the minimum of mechanical disturbance in covered containers. Lesions were cut from them, with a border of unaffected leaf surface, and mounted on glass slides on cushions of glycerine jelly. Air-currents of known velocity were then directed, at an angle of 30°, on to the pustule for 10 min. periods. In addition, vibration could be applied; this was produced by running a low-speed electric motor, fitted with an eccentric weight on the shaft, 2 ft. away from the microscope stage on which the slide was carried. For air-speeds of up to 7 m.p.h. an electric fan was used; for higher speeds, an industrial blower. The air currents produced in this way were measured by a small, vane-type anemometer. Each exposure to an air-stream was followed by a similar exposure to an air-stream of the same velocity with added vibration. Photographs were taken before and after each treatment, and enlargements made to give a total magnification of some 800 diameters, enabling individual spores to be recognized. The original photograph was then carefully traced on a thin Perspex sheet and this tracing, when superimposed on subsequent photographs in exact

register, enabled any movement of spores to be detected. Wind speeds of up to 12·5 m.p.h. were used. The frequency of the vibration was 1400/min.

With air-speeds of up to 9 m.p.h., whether or not vibration was applied to the pustule, no spore movement was ever detected, even after several periods of exposure. With winds of 12·5 m.p.h. a very small proportion of the detached spores were moved; most of these, however, were not blown away but were caught on other spore clusters to which they adhered. Pl. 15, figs. 1–3, illustrate typical results.

(2) A second series of experiments was carried out on entire detached leaves held horizontally by their petioles, air currents of known velocity being directed onto the pustule. The leaves were thus free to bend and vibrate as they would do under natural conditions. Sticky slides were exposed directly behind the pustule under treatment for 10 min. periods at each wind velocity. This was varied in 1 m.p.h. steps, from 1 to 6 m.p.h.

At the lower speeds, no detached spore could be found on the slides. At 6 m.p.h., the fluttering of the leaves in the air-current became appreciable, the surface of the pustule vibrating with an amplitude of at least 0·5 cm. Under these conditions spores were detached, and caught on the slides; it is noteworthy, however, that no individual spores were collected, but only clusters, which presumably were jerked off the leaf by the intense movement.

(3) Further trials were carried out with branches of *Coffea arabica* bearing many heavily rusted leaves. These were set up in the laboratory, and winds of known velocity directed onto them. Immediately behind the branches a rotating-rod spore-trap was placed, fixed in a horizontal position and sampling air at the rate of 116 l./min. Periods of 10 min. at each wind-velocity were used. At the higher velocities greater leaf movement than is normal in a plantation occurred, although there was not the violent flutter which resulted when the leaf was rigidly secured by its petiole. Air speeds of 2, 4 and 7 m.p.h. were used. Even at the higher speeds no rust spores were trapped.

It is therefore apparent that air-currents, whether or not accompanied by vibration, of up to 12 m.p.h., will not dislodge ripe spores of *Hemileia* from the pustule. Under conditions where rapid leaf-flutter occurs, with winds of 6 m.p.h. and over, large clusters of spores can be dislodged from the surface of the pustule by the movement, but individual spores remain unaffected.

In order to compare the behaviour of pustules of *H. vastatrix* with those of the admittedly wind-borne *Puccinia graminis*, freshly sporing pustules of the latter were brought to the laboratory, mounted on glycerine jelly in the same manner as in the experiment described above, and subjected to air-currents of known velocities. Clouds of uredospores were removed from these pustules at the lowest wind-speeds for which our equipment was calibrated.

Effect of liquid water on the pustules

The ripe uredospores comprising a rust pustule are instantly liberated from their mutual adhesion if water is applied to them. This has been observed experimentally, both by lowering hanging droplets on to the

pustule surface with great delicacy, so as to minimize lateral movements, and also by developing a droplet on the surface at a very slow rate through a fine glass needle attached to an Agla micrometer syringe. The droplets immediately become coated with individual spores, and only very rarely are groups or clusters seen. Pl. , fig. 4, illustrates spores liberated in this manner, and figs. 5 and 6 the appearance of a pustule both before and after water has been applied. It can be seen that considerable movement of uredospores has been effected.

Effect of air-movement on detached spores

Droplets of water containing spores detached from pustules were transferred respectively to leaf surfaces, to polished glass slides, and to polished metal surfaces. They were then permitted to dry, and were subjected to gradually increasing air-currents at speeds of up to 12 m.p.h., directed at an angle of 30° to their surfaces, with and without vibration. Pl. 16, figs. 9, 10, and 11, 12, shows spores on glass and metal surfaces, both before and after being subjected to winds of 12 m.p.h., combined with vibration. It can be seen that no spores have been lost or even altered in position. Results were similar on leaf surfaces.

Ripe spores detached from pustules by tapping the leaves, or by light brushing, were treated similarly. Unlike those detached by water, these usually occurred in clusters of considerable size. On a leaf surface, both the clusters and the occasional individual spores remained unaffected by winds of 12 m.p.h. combined with vibration (Pl. 16, figs. 7, 8). On glass and on metal surfaces, individual spores remained adherent while some of the larger clusters, as might have been expected, were blown away.

Rate of fall of spores of Hemileia vastatrix

Ripe spores were liberated at the upper end of a 1 m. long vertical tube, sticky slides being exposed at the bottom after varying time-intervals. Clusters, each of a dozen or so spores, reached the bottom of the tube in less than 5 sec., individual spores falling in 10–15 sec. Spores of the cereal rusts, according to Gäumann (1950), fall in still air at about 1 cm./sec. Clusters of spores of *H. vastatrix* fall at least twenty times faster, while the average rate of fall of individual spores is about ten times faster.

Discussion

It has usually been assumed that the chief method of distribution of the uredospores of *H. vastatrix* has been by wind. Butler (1918), however, although he made no comment on their probable local method of distribution, apparently discounted wind as a factor in the geographical spread of the disease, as he concluded that it must have come to Asia from its original home in Africa through human agency, by the transport of living plants or other contaminated material from island to island. No such conclusion would be necessary to explain the dispersal of the world's main cereal rusts.

It seems, however, later to have been assumed by all who have had to deal with *H. vastatrix* that the main method of dispersal was by air-borne

spores. Thus Rayner (1956) writes of them as being 'blown many hundreds of miles'. This author later (1958) expressed the view that the normal method by which the air-borne spores reach the underside of the leaf is by their eventual landing on the upper surfaces, from which they are washed by rain. The results of the experiments described here make it seem unlikely that wind plays other than a minor part in the distribution of the uredospores of *H. vastatrix*. A certain number must, of course, eventually become detached from the pustules and then become air-borne, although, as spores are rarely removed singly, the large clusters are not likely to travel far; it has been shown experimentally that the rate of fall of such clusters is more than twenty times faster than that of spores of the wind-dispersed cereal rusts. Calculations based on this rate of fall show that the clusters are likely to travel an average distance of less than 2 miles, the figure for single spores being less than 7 miles. These figures are in sharp contrast with the calculated average range of the cereal rusts, which is nearly 700 miles. Further, when spores, or clusters of spores, alight on a leaf, wind velocities of a higher order than are usual in plantations are necessary again to detach them. There thus seems to be no possibility of long-distance dispersal through the spores being blown from leaf to leaf.

Field observations over a period of 8 months have shown that at no time during this period was rust completely absent. The level, however, during January–February 1959 was extremely low, averaging less than one pustule per fifty leaves at two different sites. It is suggested that these scattered foci of infection are very probably the result of wind-blown uredospores. The subsequent build-up of the rust population to epidemic proportions can be, and usually is, extremely rapid.

It has been shown that, even under conditions when spores would be expected to be present in the air in quantity, that is, in a plantation at the height of an epidemic when the leaves are being mechanically disturbed, it is very unusual to trap any spores, even with the highly efficient equipment used; it seems most improbable, therefore, that enough inoculum can be carried by the wind to give the observed rapid build-up. Further, it has been demonstrated that uredo-pustules remain substantially unaltered after subjection to winds of a velocity and duration which rarely, if ever, occur within a plantation.

Water, however, if applied to the surface of a pustule, instantaneously releases the spores, which float singly to the surface (Pl. 15, fig. 4). It seems clear, therefore, that the build-up from the scattered and low infection found in part of the year to epidemic proportions can only be the result of spore distribution in an aqueous medium. Thus it follows that this distribution can only be effected actually during a rain-storm, and, after rain, only while the leaves are still running-wet. Spores thus distributed may, if conditions remain favourable, infect at once. If the leaves dry before germination occurs, the spores are likely to adhere under any normal conditions of leaf-flutter, and could germinate if conditions again became suitable, though drying of once-wetted spores reduces viability.

The conclusion drawn from this study therefore is that, except when rain is accompanied by winds of high velocity (which are not normally experienced in the districts of Kenya under discussion), spore distribution

in the 'build-up' phase of an epidemic can be effected only by water-splash, and therefore is likely to be confined to the same tree, or, at most, to the immediately adjacent ones. Mayne (1930), too, deduced from a study of epidemics that the build-up of an epidemic results from inoculum derived from the same block of trees, indicating 'the absence of any considerable external source of infection'.

To sum up, it seems that the distribution of *Hemileia vastatrix* spores by air takes place throughout the year, under suitable conditions, but at a very low rate, probably producing the light and even scatter of pustules that can be found at all times. The sudden increase of the disease to epidemic magnitude, however, is the result of their effective dispersal by water.

These conclusions have obvious bearing on control methods. These have in the past been based on the principle of maintaining a protective coating on the leaves, on the assumption that aerial distribution will take place in any event, and that the omnipresent uredospores will germinate and cause infection when suitable conditions permit. It now appears possible that if control is exercised during the periodic low levels of infection which occur in the course of an average annual epidemic cycle, no further treatment should be necessary. In other words, these findings suggest that rational control of *Hemileia vastatrix* might be based on elimination of inoculum at a specific time, rather than on the prevention of infection over a considerable period.

We have pleasure in acknowledging the help given by the East African Meteorological Department for information on wind-speeds, and for the loan of an anemometer, and also by Dr P. H. Gregory through his invaluable advice on spore-trapping methods and equipment.

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EXPLANATION OF PLATES 15 AND 16

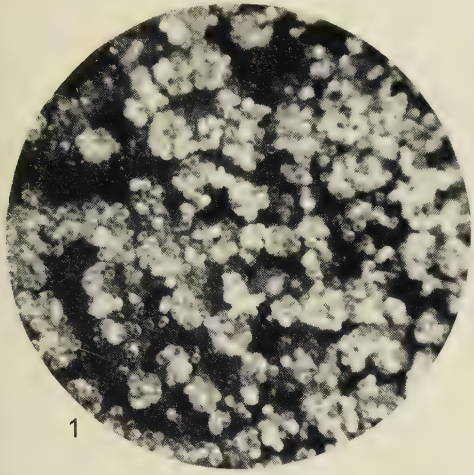
Plate 15

Fig. 1. Portion of pustule of *H. vastatrix*. × 425.

Fig. 2. The same, after 20 min. exposure to a wind of 6 m.p.h., the last 10 min. being with added vibration at a frequency of 400/min. × 425.

Fig. 3. The same, after two successive exposures to winds of 9 and 12 m.p.h., the last half of each 20 min. exposure being with added vibration. × 425.

Fig. 4. Surface of water-drop at edge of pustule showing floating spores. × 500.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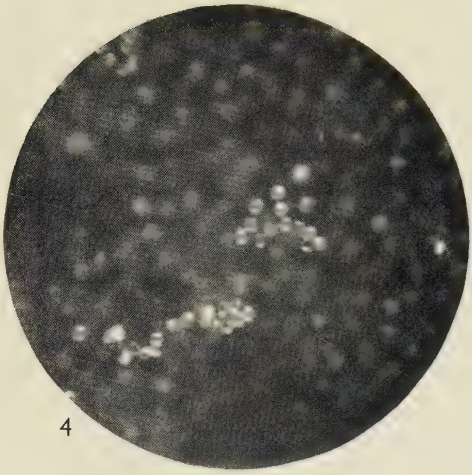
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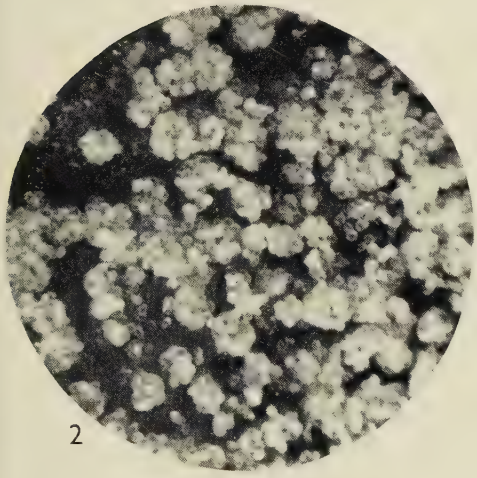
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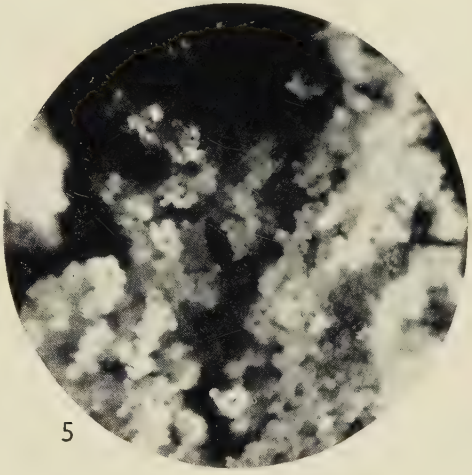
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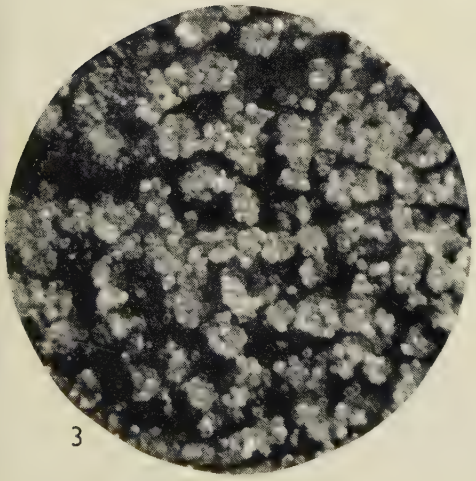
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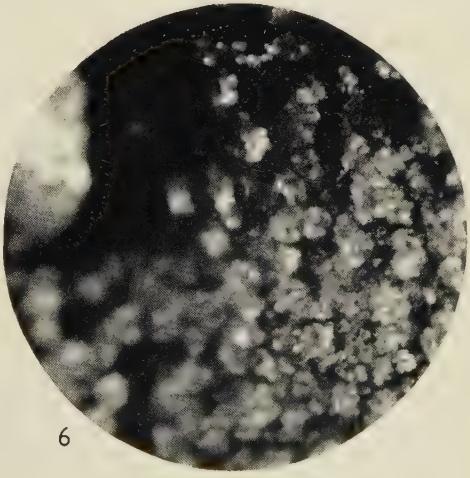
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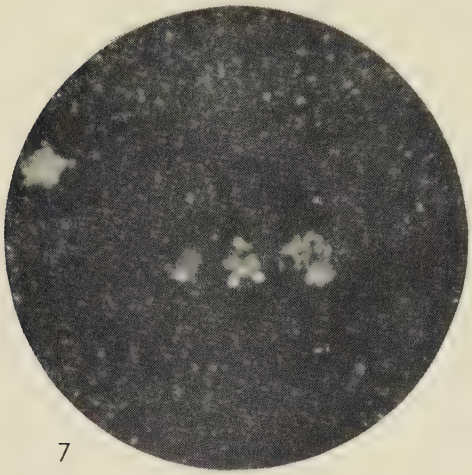

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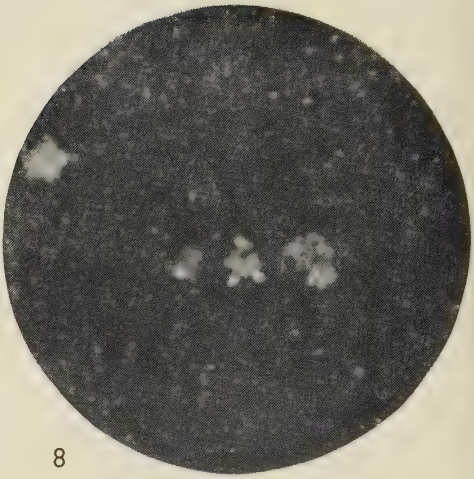

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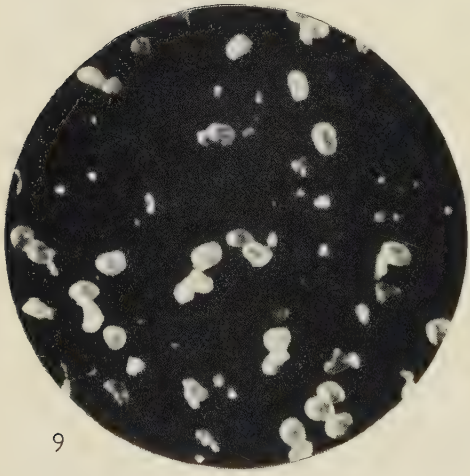

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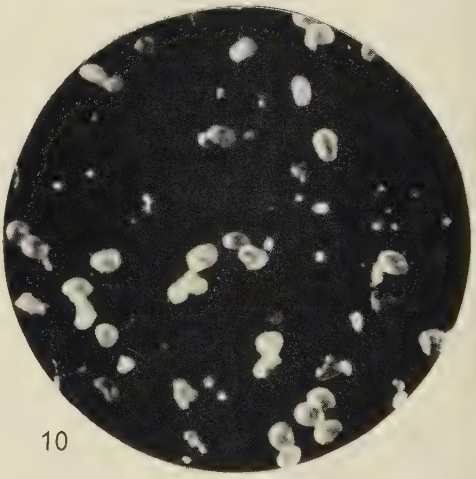

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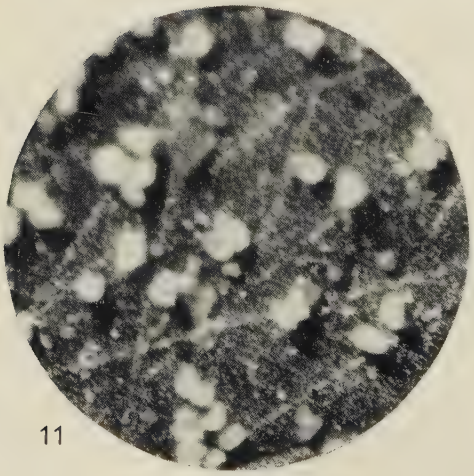

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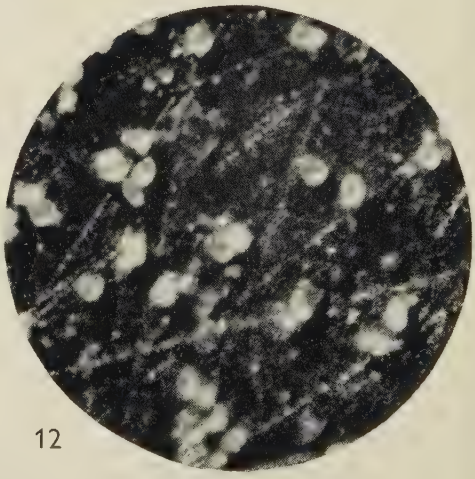

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Fig. 5. Portion of pustule showing surface incrustation of detached spores. × 425.
Fig. 6. The same, after a droplet of water has been applied and removed. The surface incrustation of mature spores has been removed, the small clusters of immature spores thus becoming visible. (The object at the left is the shank of a pin used as a marker.) × 425.

PLATE 16

Figs. 7, 8. Spores and spore clusters on a coffee leaf, before (7) and after (8) a 10 min. exposure to an air-current at 12 m.p.h. × 425.
Figs. 9, 10. Spores on a glass surface, before (9) and after (10) a 10 min. exposure to an air-current at 12 m.p.h. × 1250.
Figs. 11, 12. Spores on a metal surface, before (11) and after (12) a 10 min. exposure to an air-current at 12 m.p.h. × 1250.

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Trans. Brit. mycol. Soc. **43** (3), 516–518 (1960).

WHITE LEAF-STREAK OF RICE CAUSED BY *RAMULARIA ORYZAE* SP.NOV.

By F. C. DEIGHTON

Commonwealth Mycological Institute, Kew

and DOROTHY SHAW

Department of Agriculture, Stock and Fisheries, Port Moresby, Territory of Papua and New Guinea

(With Plate 17 and 1 Text-figure)

Ramularia oryzae sp.nov. is described, causing a white leaf-streak of *Oryza* in Papua and New Guinea, British Solomon Islands Protectorate, North Borneo, Sierra Leone and Nigeria.

A well-marked and distinctive leaf spot of cultivated rice (*Oryza sativa* L.) is common in the Territory of Papua and New Guinea, and is associated with a hitherto undescribed fungus which is described below as *Ramularia oryzae* sp.nov. 'White leaf-streak' is proposed as an apt name for the disease.

The same leaf spot and associated fungus have also been recorded on *Oryza sativa* from British Solomon Islands Protectorate, North Borneo and Northern Nigeria: it has been known for many years in Sierra Leone where, however, it has only been seen on cultivated strains (both upland and wetland) of *O. glaberrima* Steud. and on the wild perennial rice *O. barthii* A. Chev., and never on *O. sativa* even when adjacent to infected plants of *O. glaberrima* or *O. barthii*.

Mrs E. Harris tells us that at Samaru, Northern Nigeria, this disease was plentiful on a plot of G. 75 (a wetland strain of *O. sativa* from British Guiana) in November 1957, but did not appear in 1958 on the same plot planted with seed from the 1957 crop.

Attempts to grow the fungus in culture have so far been unsuccessful.

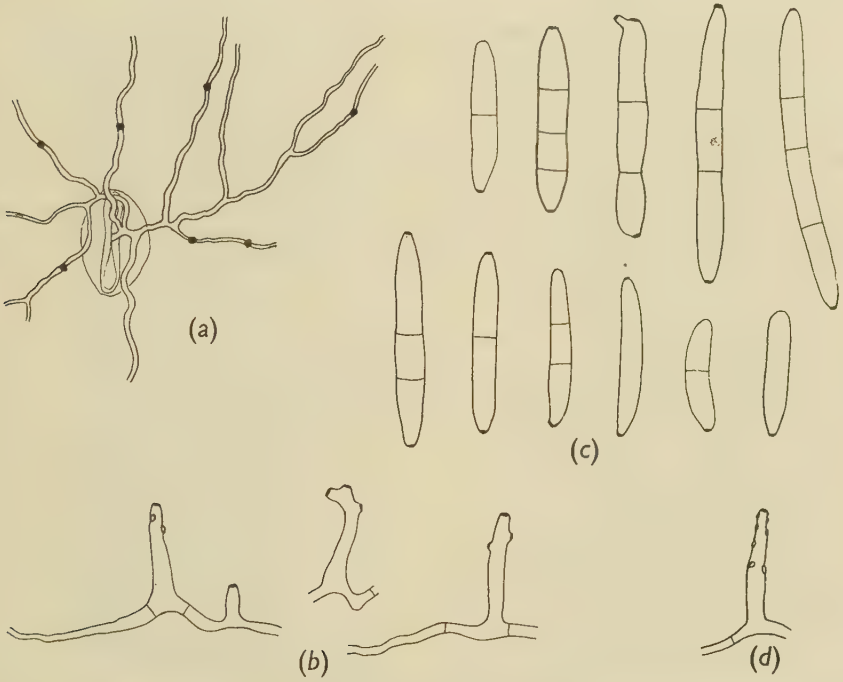
Ramularia oryzae Deighton & Shaw, sp.nov.

Maculae oblongo-lineares, 1–2·5 mm. (raro 3 mm.) longae, 0·5 mm. latae, albidae vel griseo-albidae, margine brunneo tenuissimo vel interdum usque ad 0·5 mm. lato cinctae. *Mycelium primarium* immersum, inter et intra cellulas folii penetrans, ex hyphis septatis, ramosis, 1–2 μ latis compositum, et hyphas 1–3, 2–3 μ latas, per stomata emergentes efficiens. Stroma nullum. *Mycelium secundarium* superficiale, amphigenum, ex hyphis per stomata emergentibus oriundum, repens, hyalinum, septatum, leniter sinuosum, 0·75–1·25 μ (raro 2 μ) latum, prope stoma plurime ramosum, deinde rarius ramosum et reticulum laxum efficiens. *Conidiophora* amphigena, ex mycelio superficiali oriunda, plus minusve distantia, hyalina, erecta, continua, recta, 4–20 μ longa, 1·75–2·25 μ lata. Cicatrices conidiales conspicuae, 0·75–1·25 μ latae, primo terminales, denique in positionem lateralem submotae et saepe plus minusve prominentes. *Conidia* hyalina, cylindrica, recta, utrinque leniter attenuata, catenata, 0–3-septata (plerumque 1-septata),

hilo conspicuo incrassatoque 0·75–1·25 μ lato praedita, pariete minute irregulariterque asperato, 15–40 × 2·5–4·75 μ, saepius 20–25 μ longa.

Habitat in foliis vivis *Oryzae sativae* Linn., New Guinea: Madang, 15. III. 1958, leg. D. Shaw, Herb. I.M.I. 73536, typus.

Leaf spot visible on both surfaces, oblong-linear, 1–2·5 mm. (more rarely 3 mm.) long, 0·5 mm. wide, white or greyish white, surrounded by a brown margin which is usually very narrow but sometimes up to 0·5 mm. wide, sometimes with a rather more diffuse brown area outside the margin of the spot. Younger lesions may show a white streak only on the upper leaf surface, many of the streaks on the lower surface being brown all over. The



Text-fig. 1. *a*, Diagrammatic drawing showing branching of the repent superficial hyphae (septa not shown) after emergence from a stoma: the position of the conidiophores is shown by black circles; × 580. *b*, Conidiophores and *c*, conidia; × 1000. From the type, I.M.I. 73536. *d*, A conidiophore showing eight lateral scars; × 1000. From I.M.I. 1318, on *Oryza barthii*.

lesions are usually numerous and on heavily infected leaves may be contiguous, though each remains clearly delimited by its own narrow brown margin. The spot is limited laterally by the veins of the leaf.

Internal mycelium consisting of hyaline branched septate hyphae 1–2 μ wide, ramifying between, and penetrating and killing, the cells of the mesophyll of the leaf. Stroma none. External mycelium amphigenous, developing from 1 to 3 hyphal threads, 2–3 μ wide, which emerge through a stoma, hyaline, septate, branched several times immediately after emergence from the stoma and sparingly branched thereafter, slightly sinuous, long (up to 100 μ or more), creeping over the leaf surface, 0·75–1·25 μ wide (less often

2μ wide), bearing the rather distant erect short conidiophores. The external mycelium forms a thin network over the whole surface of the lesion, and it is difficult to trace the hyphae arising from each stoma throughout their entire length. Occasionally, a conidiophore is borne on a branch arising from near the stoma, but the conidiophores are usually produced on the repent hyphae at some distance from the stoma.

Conidiophores amphigenous, hyaline, continuous, erect, straight, 4–20μ long, 1·75–2·25μ wide, with conspicuous flat often slightly prominent conidial scars 0·75–1·25μ across, first terminal, later pushed to one side by continued growth of the conidiophore and lying more or less flat against the sides of the conidiophore. Up to eight lateral scars have been seen on one conidiophore in the collection (I.M.I. 1318) on *O. barthii*, but most collections show only one to two lateral scars in addition to the terminal scar.

Conidia hyaline, cylindric, straight, slightly tapered at each end, the wall minutely and irregularly roughened (but not wrinkled, spinulose or warted), catenate, sometimes forming branched chains, 0–3-septate (mostly 1-septate), with a conspicuous thickened hilum 0·75–1·25μ wide, 15–40 × 2·5–4·75μ, most frequently 20–25μ long.

On *O. sativa*, Territory of Papua and New Guinea, British Solomon Islands Protectorate, North Borneo and Northern Nigeria; and on *O. glaberrima* and *O. barthii* in Sierra Leone.

Specimens examined

(Herb. I.M.I. accession numbers are given in brackets after each collection. F.C.D. = F. C. Deighton; D.S. = D. Shaw.)

On *O. barthii*: Sierra Leone: Rokupr (Magbema), July 1939, F.C.D. (1318).

On *O. glaberrima*: Sierra Leone: Masasa (Biriwa), Oct. 1928, R. R. Glanville (1319); Njala (Kori), Nov. 1933, F.C.D. (10379).

On *O. sativa*: New Guinea: Amele Madang, Apr. 1956, G. Keleny (73541); Bainyik, Feb. 1956, J. R. Vicary (73539); Balupwine near Bainyik, Feb. 1956, J. R. Vicary (73540); Danben, Nov. 1955, D.S. (73538); Erap, July 1956, D.S. (73542); Josephstaal and Annaberg, Dec. 1957, P. G. Owens (73546); Madang, Mar. 1953, J. R. Vicary, Herb. SYD 895 (51930); Madang, 15 Mar., 1958, D.S. (73536), type; Madang, Mar. 1958, D.S. (73537).

Papua: Jimmy's, Kapurahambo near Popondetta, Feb. 1957, J. Healy (73543); Kapurahambo near Popondetta, Apr. 1957, D.S. (73544); Mainahano Catholic Mission, near Epo, May 1957, D.S. (73545); Epo, June 1959, D.S. (77633).

British Solomon Islands Protectorate: Maleai, Shortland Islands, May 1959, A. Johnston (77134).

North Borneo: Keningan agric. Station, Feb. 1956, R. H. Forster (63300).

Northern Nigeria: Samaru (Zaria), Nov. 1957, E. Harris (71465).

Explanation of Plate 17

Figs. 1, 2. *Ramularia oryzae*: photographs of lesions on the upper leaf surface of *Oryza sativa*, from fresh material; × 2 (approx.). From I.M.I. 77633, collected in Papua.

(*Accepted for publication* 19 *August* 1959)

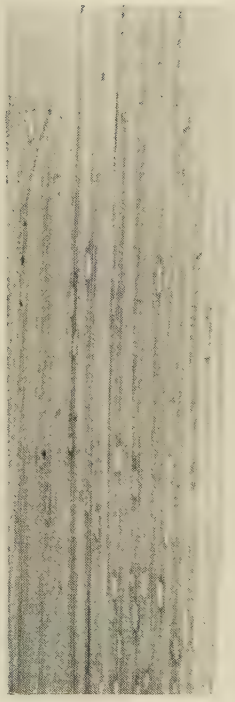


Fig. 1

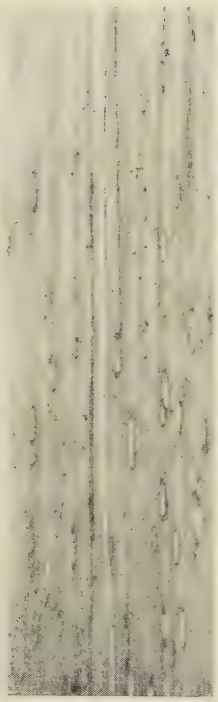


Fig. 2

(*Facing p.* 518)

Trans. Brit. mycol. Soc. **43** (3), 519–522 (1960).

STUDIES ON *DIDYMELLA LYCOPERSICI* KLEB., THE CAUSAL FUNGUS OF STEM ROT DISEASE OF TOMATOES

By DOROTHY E. KNIGHT (née FISHER)

Formerly of the National Vegetable Research Station, Wellesbourne, Warwick

Isolates of pycnidial fungi from lesions on tomato stems and fruit could be grouped into those which were pathogenic to stems and fruit and those which were pathogenic to fruit only. The fungi in the first group produced a proportion of bicellular spores and agreed with the description of *Diplodina lycopersici*, the imperfect stage of *Didymella lycopersici*. Those in the second group mostly conformed to the description of *Phoma destructiva* and produced no bicellular spores, although a few (isolated from stems) appeared to be weakly pathogenic forms of *Diplodina*.

Studies on single ascospore cultures of *Didymella lycopersici* suggest that the fungus is heterothallic.

Studies on isolates of the pathogen

During the course of investigations on the stem rot disease of outdoor tomatoes (Knight & Keyworth, 1960) a collection was made of seventy-three isolates of pycnidial fungi from lesions on tomato stems and fruits from different parts of Britain. They were grown on modified Coon's agar (maltose, 7 g.; KNO_3, 2 g.; KH_2PO_4, 2·7 g.; $MgSO_4$, 1·2 g.; agar, 20 g.; water, 1 l.) and stored under medicinal paraffin until required. The pathogenicity of the isolates was determined by inoculating them into stems of 12-week-old tomato plants growing in pots in a glasshouse, and into the calyx scars of tomato fruits, which were incubated individually in tins. The results of these tests showed that the isolates could be classified into two groups.

Group 1. *Isolates which attacked both stems and fruit.* Fifty-one of the seventy-three isolates were in this group, thirty-seven being obtained from stems and fourteen from fruit. The fruit lesions from which the latter isolates came were all around the calyx ends of the fruit. All the isolates were of approximately equal pathogenicity both on stems and fruit.

Group 2. *Isolates which attacked fruit only.* (*a*) *Obtained from fruit.* There were fifteen such isolates, nine of which came from lesions at the calyx end and six from lesions on the sides of fruit. They attacked the fruit readily, but in three separate tests they all proved to be non-pathogenic to stems.

(*b*) *Obtained from stems.* The seven isolates in this group were obtained from lesions of normal appearance on stems, but when inoculated into stems they proved to be non-pathogenic. Two of them readily infected fruit but the other five were only weakly pathogenic. No isolates were obtained which attacked stems but did not attack fruit, and it appeared that the fungus normally associated with the stem rot phase of the disease was also capable of attacking tomato fruit. However, the reverse was not necessarily the case.

Cultural characters

Selected isolates from group 1 and group 2*a* were grown on three different types of nutrient agar, with the object of determining whether those that were pathogenic to stems differed morphologically from those that were pathogenic only to fruit. Measurements were made of the growth rates of the cultures, and of the sizes of the pycnidia and pycnospores; observations were also made of the appearance of the cultures and the shape and septation of the pycnospores.

There were no marked differences between the growth rates of the cultures in the two groups, nor did they differ consistently in appearance, so that they could not be classified on cultural characters. Moreover, the pycnidial and spore sizes varied so widely (even within one culture) that no differences were apparent between the two groups. It was noted, however, that the cultures in group 1 produced a small number of bilobed or bicellular spores, whereas those in group 2*a* did not. Further measurements and observations were therefore made of pycnidia and pycnospores produced by the various isolates *in vivo* on tomato stems and/or fruits. The mean sizes of the pycnidia and pycnospores were again approximately the same for all isolates, but those in group 1 produced a higher proportion of bilobed or bicellular spores than they had done on agar. None of the isolates in group 2*a* produced bicellular spores *in vivo* and this criterion thus provided a means of distinguishing the two types. Examples are given in Table 1.

Table 1. *Isolates of pycnidial fungi from lesions on tomato stems and fruits*

(Data on pycnospore size and shape *in vivo*.)

Group	No. of isolate	Sources	Grown on	Pycnospore dimensions (μ) Length Range	Length Mean	Breadth Range	Breadth Mean	Pycnospore form* R	O	C	B	S
1. Pathogenic to stems and fruits	1	Stem (Alcester, Warwickshire)	Stem	2·4–11·9	7·2	2·4–4·7	3·2	0	14	64	14	8
		Ascospore culture	Fruit	2·4–11·9	8·2	2·4–4·7	3·6	4	12	62	10	12
	2	Fruit (Ash, Kent)	Stem	2·4–11·9	7·7	2·4–4·7	3·4	10	18	50	15	7
			Fruit	2·4–11·9	6·7	2·4–4·7	3·2	8	24	59	8	1
2*a*. Pathogenic to fruits only	3	Fruit (Long Marston, Warwickshire)	Fruit	3·6– 8·3	5·7	2·4–3·6	2·8	5	9	86	0	0
	4	Fruit (Harvington, Worcestershire)	Fruit	2·4– 8·3	5·8	1·2–3·6	2·3	4	5	91	0	0

* Pycnospore forms classified as follows: R = round; O = oval; C = cylindrical; B = bilobed, non-septate; S = bilobed, uniseptate.

The group 1 isolates shown in Table 1 produced up to 12 % of bicellular spores and also up to 15 % of bilobed spores. Neither type of spore was found in the isolates in group 2*a*. The spores of these latter isolates also included a larger proportion of cylindrical forms. The pathogenicity and spore characters *in vivo* of the isolates in these two groups agree with those

of *Diplodina lycopersici* (Cooke) Hollos emend. Brooks & Searle, and *Phoma destructiva* Plowr., respectively (Brooks & Searle, 1921). *Diplodina lycopersici* was regarded by Klebahn (1921) as the imperfect stage of *Didymella lycopersici* Kleb., and in this connexion it may be noted that isolate no. 1 in the present study was derived from a single ascospore obtained from a perithecium of *D. lycopersici* (see below).

It was not possible to make a comparable series of observations on isolates in group 2*b* (obtained from stem lesions, but infecting fruit only). Some of them were found, however, to produce a few bicellular spores *in vivo* and thus they may have been degenerate forms of the types in group 1. All of them grew more slowly in culture than did the group 1 isolates.

It appears from the studies described here that the majority of the isolates from stem lesions grew in culture into the imperfect stage of *D. lycopersici* and were characterized by their pathogenicity to tomato stems, and by the production of a proportion of bi-cellular pycnospores *in vivo*. Some isolates from fruit were also of this type. Other isolates from fruit, which would not infect stems and did not form bicellular spores, conformed to the description of *Phoma destructiva*. The isolates from stems which did not re-infect stems and were similar to the imperfect stage of *D. lycopersici* may have lost their pathogenicity in culture, or they may have been mutants which attacked fruit only. The latter possibility might explain the observation that typical Didymella fruit rot occasionally occurs on growers' holdings where there is little or no stem rot. The above conclusions are in agreement with those of Phillips (1955).

Perithecial stage of *Didymella lycopersici* Kleb.

Klebahn (1921) first described the perithecial stage of *Didymella lycopersici* and named the fungus. Perithecia were first seen in Britain by Hickman (1944), who found them on the rotting cortical tissue at the bases of old tomato stems in the field. They were seen by the author in November 1954 on similar material at Alcester, Warwickshire (Fisher, 1955), and again in 1956. The perithecia were used in a study of single-ascospore cultures of the fungus.

The primary object of this study was to grow cultures from the eight spores within one ascus to determine whether the fungus was homothallic or heterothallic. Because of their small size (70–95 μ long × 9–10 μ wide), it was extremely difficult to select single asci and to dissect out the spores. Instead the spores were allowed to shoot up on to 3 % plain filtered agar on slides suspended over pieces of tomato tissue bearing perithecia. Groups of eight spores lying close together were found on the agar after 16 hr. and it was assumed that each group had come from a single ascus. The spores in a suitable group were separated by means of fine needles and transferred individually to plates of modified Coon's agar, using a disc cutter attached to the microscope (La Rue, 1920).

It was found that the eight colonies grown from single spores from one ascus could be grouped on their appearance in culture into four distinct pairs. Two of the pairs were dark-grey and two were light-grey in colour. This grouping suggests that *D. lycopersici* is a heterothallic ascomycete.

Attempts were made to test this possibility by determining whether various pairings of the single-spore cultures would give rise to perithecia. The methods used were based on conditions shown by Hawker (1950) to be necessary for perithecial formation in some other fungi and inoculations were also made into stems of tomato plants and on to pieces of autoclaved hop stem. Perithecia were formed only on the hop stems and they occurred when certain pairs of cultures were grown together on the stems and maintained at 57° F. They were produced in only five of the twenty-eight possible combinations of the eight different isolates in pairs. None was found where pairs having a similar appearance in culture (or two cultures of the same isolate) were combined. This result suggests that the fungus is heterothallic, and that the heterothallism is associated with differences in appearance of the cultures. This conclusion must be accepted with reserve, however, until the discovery of more suitable conditions for the production of perithecia enables a more complete series of tests to be made.

The work described above formed part of a thesis approved for the degree of Doctor of Philosophy in the University of London.

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Trans. Brit. mycol. Soc. **43** (3), 523–524 (1960).

ROBILLARDA PHRAGMITIS CUNNELL IN ESTUARINE WATERS

By T. W. JOHNSON and GILBERT C. HUGHES

Department of Botany, Duke University, Durham, and the Marine Laboratory, Beaufort, North Carolina

The sphaeropsidaceous fungus *Robillarda phragmitis* Cunnell was collected on wood submerged in the fresh and brackish water sectors of a North Carolina estuary. The fungus also occurs on *Phragmites* culms in the upper reaches of the same estuary.

Robillarda phragmitis Cunnell (1958), originally described from a dead culm of *Phragmites communis* Trin. submerged in fresh water near Staines, Middlesex, has been collected on panels of pine (*Pinus* spp.) and yellow poplar (*Liriodendron tulipifera* L.) submerged in an estuary on the North Carolina coast. The wood panels were used in an ecological study of the estuarine distribution of lignicolous fungi. In 1957 *R. phragmitis* was found on a pine panel submerged for 1 month in the non-saline part of the estuary and on a yellow poplar panel submerged for the same period in the brackish part (3–16‰ salinity) of the same estuary. It was also noted in culms of *P. communis* at the edge of a river emptying into the main course of the estuary. Again, in 1959, *R. phragmitis* was collected on a pine panel, which had been submerged in fresh water in the estuary for 2 weeks and then incubated (by the method described by Johnson, Ferchau & Gold, 1959) for 1 week.

The material from *Phragmites* is identical in all morphological respects with the collection described by Cunnell (1958), the conidia being well within the size range reported. On the pine and poplar panels, however, the pycnidia are very pale brown and always superficial instead of yellow brown and innate. While the fructifications are ellipsoid, they are not irregularly so (Cunnell, 1958, fig. 1*e,f*). The ostiole is large, round, and the cells surrounding it are either concolorous with the rest of the pycnidium or dark brown. Conidia from the fructifications on wood in fresh water are identical with those from pycnidia in *Phragmites*, but those from wood submerged in brackish water have shorter appendages. The predominating appendage length is 8–12 μ (max. 19 μ).

Two aspects of the North Carolina collections may be emphasized. First, *R. phragmitis* is not limited to *Phragmites*; the fungus occurs as well on wood. Secondly, *R. phragmitis* can develop in saline waters and is not restricted to fresh water. While we cannot account for the ability of the fungus to tolerate saline waters, other fungi commonly considered to be non-aquatic (*Cladosporium herbarum* Link ex Fr., *Stemphylium lanuginosum* Harz, *Pullularia pullulans* Berkhout, and *Chaetomium bostrychodes* Zopf, among others) are also known to occur on wood submerged in sea water (Siepmann & Johnson, unpublished observations). The occurrence of a 'fresh water'

fungus, such as *R. phragmitis*, in salt water and on wood suggests that the use of habitat and substratum type in descriptive mycology could well be de-emphasized.

Support of the National Science Foundation, through Grant G-7178, is gratefully acknowledged.

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Trans. Brit. mycol. Soc. **43** (3), 525–528 (1960).

AN APPARATUS FOR STUDYING HYGROSCOPIC RESPONSES IN FUNGAL CONIDIOPHORES

By W. R. JARVIS

Scottish Horticultural Research Institute, Invergowrie, Dundee

(With Plate 18 and 1 Text-figure)

A simple, inexpensive apparatus is described in which fungal cultures can be observed in atmospheres of different relative humidity. Eight cultures can be moved rapidly and simultaneously from one atmosphere to another without exposure to the air of the laboratory. The method is discussed in relation to the limitations of established methods of investigating hygroscopic responses in fungi.

Observations on the reactions of the conidiophores to changes in relative humidity and on hygroscopic mechanisms of spore release necessitate the rapid transfer of cultures between different controlled environments. A number of methods of achieving this have been described. These fall broadly into two groups, the first requiring transfer of the culture between two vessels, such as Petri dishes, and the second the replacement of air in a single vessel.

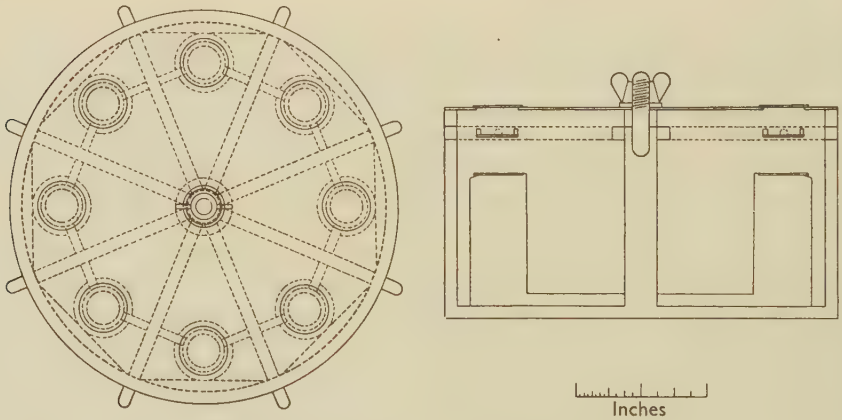
An example of the first group is given by Cruikshank (1958). Sporulating material of *Peronospora tabacina* on leaf discs was fixed inside Petri dish lids by means of agar discs, and transferred to new atmospheres by rapidly exchanging the lids with those of Petri dishes containing different humidifying liquids. Two serious disadvantages are inherent in this method, namely that the conidiophores are exposed to the air of the laboratory during transfer, and that, owing to the small volume of the Petri dishes, considerable changes in the contained atmosphere must be caused by the rapid movements of the operator, with consequent slow recovery to the required humidity. Observations on *Botrytis cinerea* in the present apparatus have shown that hygroscopic responses may occur within a few seconds of exposure to a new environment.

Methods involving air replacement have the advantage that the fungus under observation is not exposed to the air of the laboratory, and that temperature, which profoundly affects relative humidity, is more easily controlled. Pinckard (1942) kept conidiophores of *Peronospora tabacina* under observation in a specially constructed van Tieghem cell fitted with side arms. Air was bubbled through humidifying liquids and passed slowly into the cell. Since it is usually desirable to eliminate the mechanical effects of air currents, the rate of flow must be low, and so the disadvantage of slow atmosphere replacement is introduced.

The choice of humidifying liquids to be used in work with fungi has received much attention. Thom & Shaw (1915) and Stevens (1916) first used solutions of sulphuric acid at the concentrations given in tables by

Hastings (1909). Although they have since been widely used, sulphuric acid solutions have certain serious disadvantages as humidifying agents. Thut (1938) suggested that SO_3 may be released from the solution to redissolve in water films on plant surfaces, lowering the pH sufficiently at least to affect stomatal guard cell movement, and Zwölfer (1932) showed that SO_2 was also given off, particularly if the solution was contaminated with organic particles. Later workers, for example Clayton (1942) and Wahl (1946), have used saturated solutions of various salts and various sucrose solutions, the concentrations of which were based on figures given by Spencer (1926).

Carson (1931) and Hopp (1936), however, pointed out that the concentrations in saturated salt solutions and sulphuric acid solutions are very sensitive to temperature changes, and Carson suggested the use of glycerol solutions which are relatively insensitive. As with any unsaturated solution, however, care must be taken to minimize dilution which may result



Text-fig. 1. Plan and (right) vertical section of the apparatus.

from water exchange between the organism, agar, air and solution. Additional advantages of glycerol solutions are that they do not give off volatile substances and that they can be used over a wide range of relative humidity values. They have been used successfully for work with fungi by Cruikshank (1958), Cruikshank & Müller (1957) and in the present work.

In addition to the objections to the use of sulphuric acid and saturated salt solutions already noted, Vernon & Whitby (1931*b*) have pointed out that, in air replacement methods, air bubbled through water gives an atmosphere of only approximately 80% R.H., not saturated as is often supposed, so that the method of Seth (1931) for quantitatively mixing 'saturated' and 'dry' air must be used with caution. Buxton (1931) has described a method of using sulphuric acid which eliminates many of the objections, and an improved method of quantitatively mixing 'dry' and 'saturated' air streams has been described by Vernon & Whitby (1931*a*).

In general, the method of replacing the atmosphere round a fungal

culture is too slow and imprecise to be of value in observing the rapid reactions of many hygroscopic conidiophores, and a method of almost instantaneously transferring cultures to new atmospheres without exposure to the air of the laboratory is now described.

The apparatus is easily and cheaply constructed in Perspex and is compact enough to be kept in an incubator and to be used on a microscope stage. A cylinder 6 in. in diameter and $3\frac{1}{4}$ in. high is divided vertically into eight equal sectors (Pl. 18; Text-fig. 1). The vertical walls are continuous through a shallow lid and a shallow wheel-like structure immediately below the lid. The wheel is free to rotate about the central axis, and is provided with lugs on the circumference, but the lid is fixed by locating pins with respect to the main body.

Fungal cultures on agar or on leaf discs floating in mannitol solutions (Cruikshank, 1958) are contained in shallow Perspex pans mounted between the spokes of the wheel, and immediately above each pan a circular hole is cut in the lid over which is sealed a $\frac{3}{4}$ in. cover-glass for microscopic observation. The whole wheel may also be turned over so that the pans face downwards; the purpose of this is to facilitate the collection of released spores for counting, and, to this end, $\frac{3}{4}$ in. cover-glasses coated with glycerine jelly are supported in the recessed tops of removable, vertical Perspex columns. The pans containing the cultures are made small so that the surface areas of agar, mannitol solution or mycelium are kept small in relation to the surface area of humidifying liquids and to the volume of the surrounding air. The precautions noted by Hopp (1936) are thus met as far as seems practicable. All sliding surfaces are ground and lubricated with petroleum jelly or silicone grease.

The humidifying liquids are glycerol solutions (Carson, 1931); using different concentrations in the various sectors, fungal cultures may be transferred rapidly to new atmospheres, with the concomitant transfer of minimal volumes of air. The multiple chambers also enable some of the cultures to be transferred as controls to replicate atmospheres. The apparatus largely obviates the risk of mechanical shock, which Cruikshank (1958) has demonstrated to release spores of *Peronospora tabacina*, and which also releases spores of *Botrytis cinerea*.

Field observations on the air spora of raspberry plantations (Jarvis, 1957 *et seq.*) have shown that maximal spore release by *B. cinerea* occurs when the relative humidity is rising or falling at a fast rate between the approximate limits of 85 and 65 %. Experiments with the present apparatus have confirmed that vigorous hygroscopic movements of the conidiophores occur with changes of 5 % within this range. It has not been possible, however, to reproduce spore release on the massive scale observed in the field, and the mechanism of spore release is being further investigated.

Grateful acknowledgement is made of the assistance of Mr A. J. Renwick, who constructed the apparatus, and of Mr J. Sunderland who took the photographs. The author is also indebted to Dr A. R. Wilson for helpful discussion and criticism.

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EXPLANATION OF PLATE 18

The assembled apparatus with (below) its component parts.

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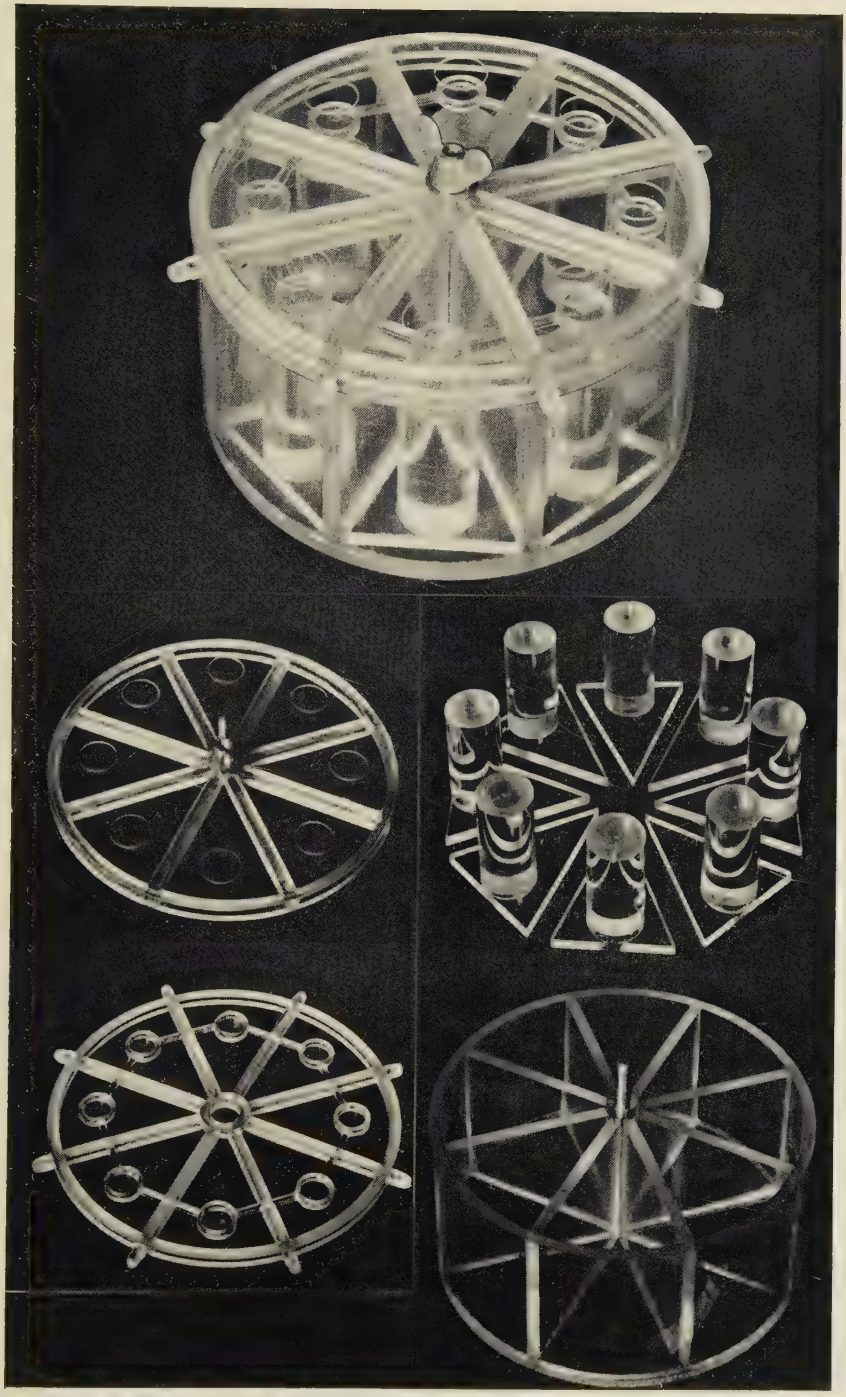

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SAPROPHYTIC ACTIVITY OF *PYTHIUM MAMILLATUM* IN SOILS

I. INFLUENCE OF SUBSTRATE COMPOSITION AND SOIL ENVIRONMENT

By R. BARTON*

Department of Cryptogamic Botany, University of Manchester

Saprophytic colonization of organic material in soil by *Pythium mamillatum* was shown to depend on the presence of a carbon source within the substrate, but the presence of nitrogen did not seem to affect colonization. Too high a concentration of such a carbon source in the substrate tended to reduce colonization by *P. mamillatum*, this being ascribed to an antagonistic effect of other soil micro-organisms. Substrates maintained at low pH (3–4) were not colonized by this fungus. High soil moisture content, relatively low temperature, reaction between pH 5·5 and 7·0, and fine soil texture were found optimum for saprophytic colonization by *P. mamillatum*.

As a typical sugar fungus (Barton, 1958), *Pythium mamillatum* Meurs belongs to that substrate group of soil fungi active in the primary decomposition of organic material in soil, in which the more easily decomposable components are utilized in metabolism. It is not yet clear exactly what type of chemical substances may be included in this category, nor indeed whether different sugar fungi differ in their requirements. However, it might be expected that a sugar fungus would, during saprophytic colonization, respond differently towards substrates differing in their content of simple organic substances. The results of an investigation of the influence of substrate composition on saprophytic colonization by *P. mamillatum* are presented in this paper. By using artificial substrates in the form of wood fragments amended with known nutrients, it was possible to determine accurately the conditions in the substrate necessary for colonization. In addition, factors external to the substrate likely to influence saprophytic colonization by *P. mamillatum*, i.e. soil conditions, have also been studied, and the results are here presented.

Materials and methods

The soils used, soils A and B, have been referred to previously (Barton, 1958). Soil A was a Lancashire garden loam, pH 5·4, and soil B was from the floor of a deciduous wood, pH 3·7. Prior to each experiment, the required soil was allowed to air-dry on a glasshouse bench, this taking 7–14 days. The calculated amount of soil was then weighed out and sufficient tap water was added to bring it to a moisture content of 50 % moisture-holding capacity (m.h.c.).

* Present address: Department of Biophysics, King's College, London, W.C. 2.

Three different types of organic material were selected for experiment, representing a wide range of substrates likely to be found in soils. Material used to represent fresh organic substrates was tissue from the interior of a turnip storage root, cut approximately into 4 mm. cubes, and sterilized by exposure to propylene oxide vapour for 24 hr. (Hansen & Snyder, 1947). Materials representing substrates containing less easily decomposable components were provided by dead oak twigs and leaves, recently shed from the tree, and collected from over the site of soil B. The leaves were cut into pieces about 5 mm. square, and the wood (after removal of the bark) into cubes of 2–3 mm., before autoclaving at 15 lb./sq.in. pressure for 20 min. In those experiments where wood fragments were impregnated with nutrient substances, the fragments were autoclaved in the requisite concentration of the amendment.

The isolate of *P. mamillatum* employed in these experiments was originally obtained from soil A. Inoculum for mixing with soil was prepared by growing the fungus for 2 weeks on a moistened 3 % (w/w) mixture of maizemeal with sand.

Saprophytic colonization of organic material in soil by *P. mamillatum* was assessed quantitatively by means of a technique based on the soil-baiting method of E. J. Butler (1907), and the 'Cambridge method' of F. C. Butler (1953) and Lucas (1955). Twenty-five fragments of organic material were placed in 50 g. test soil prepared at 50 % m.h.c. in a plugged 100 ml. conical flask. For each experimental treatment three replicates prepared in this way were always set up. The flasks were incubated for 3 days on a laboratory bench (temperature 18–22° C.). The fragments were then removed, wrapped in a square of muslin, and taken through six changes of distilled water before plating on shallow plates of oat agar. After 3 days at 20° C., when the colonizing organisms had grown out from the fragments, the plates were flooded with formalin/alcohol (10 vol. formalin:90 vol. 70 % alcohol). This fixed the organisms on the plates so that recording could be undertaken at leisure. The plates were examined under a low magnification of the microscope, and those fragments which were obvious centres of oospore production were considered to have been colonized by *P. mamillatum*. The spiny oogonium walls of this fungus facilitated its identification.

Colonization of different organic materials in soils A and B

Sand/maizemeal inoculum of *P. mamillatum* was mixed respectively with soils A and B at 50 % m.h.c. to give the following soil:inoculum ratios: 100:0, 95:5, 90:10, 75:25, 50:50, 25:75. Lots of 50 g. were put into each of nine 100 ml. flasks for each mixture. Twenty-five sterilized pieces of turnip were placed in three of these, twenty-five sterilized pieces of wood in another three, and twenty-five pieces of leaf in the remainder. The percentage of fragments of organic material colonized by *P. mamillatum* was determined for the individual treatments after 3 days' incubation (Table 1).

Comparing colonization of the three organic substrates in soils A and B, it is evident that in the latter soil, which contains no native population of

P. mamillatum, there is much less saprophytic colonization by *P. mamillatum*, even allowing for the small native population of the fungus contained in soil A. These results thus confirm the findings of Barton (1958) with these same soils. There is also a clear distinction in the ability of *P. mamillatum* to colonize the different organic materials investigated, this being evident in both soils. Turnip pieces, representing fresh organic substrates such as might be made available by green manuring, or other agricultural practices, were colonized in greater quantity than were wood or leaf fragments. Leaf pieces presented the next best substrate for colonization, while wood fragments, containing relatively few easily decomposable components, did not become colonized in soil A from the natural level of inoculum. These results indicate that colonization of organic substrates in soil depends partly on the soil environment, but also, and to a large extent, upon the nature of the substrate, those containing quantities of easily decomposable components being more readily colonized. This is in keeping with the known status of *P. mamillatum* in soil as a saprophytic sugar fungus.

Table 1. *Percentage colonization of different substrates in soils A and B*

Percentage inoculum added	Turnip				Wood				Leaf			
	Replicate flasks				Replicate flasks				Replicate flasks			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
					Soil A							
0	77	85	71	78	0	0	0	0	21	16	9	15
5	82	91	90	88	32	48	54	45	45	85	57	59
10	91	78	95	88	75	50	50	58	86	89	92	89
25	84	84	93	87	80	73	67	73	90	92	95	92
50	100	100	95	98	85	95	100	93	91	100	92	94
75	100	100	100	100	100	100	100	100	100	100	100	100
					Soil B							
0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
25	13	13	35	20	0	0	0	0	0	0	0	0
50	45	50	56	50	0	0	0	0	0	0	0	0
75	93	100	100	97	14	9	25	16	9	5	9	8

Nature of the substrate

Carbohydrates

Wood fragments were autoclaved in 2 % (w/v) solutions of the following carbohydrates: xylose, glucose, mannose, sucrose, maltose, raffinose, dextrin, starch, pectin, and distilled water as control. It can be assumed that the small wood fragments were thoroughly penetrated by the water-soluble substances used here, but penetration by the colloidal substances, i.e. starch and pectin, would be severely restricted. However, since it was the aim of this experiment to show an all-or-nothing effect, the presence of a certain proportion of substance on, or in, the wood was all that was required. Six flasks were set up for each nutrient treatment, three receiving 50 g. natural soil A and the remainder receiving 50 g. of an 85 : 15 mixture of soil A and maizemeal-sand inoculum of *P. mamillatum*. Natural soil A

contained a small native population of *P. mamillatum* and is referred to, in Table 2 and subsequently, as 'natural inoculum', whilst the soil receiving 15 % maizemeal-sand inoculum is designated as '15 % inoculum'. Twenty-five of the sterilized and appropriately amended wood fragments were added to each flask, the percentage colonized by *P. mamillatum* being ascertained after 3 days' incubation (Table 2).

All the carbohydrates tested in this experiment, except xylose and pectin, promoted a percentage of saprophytic colonization higher than that in the control fragments of wood that had received water alone. It is interesting in this respect that the complex polysaccharides, starch and dextrin, are available to this sugar fungus. Experiments to determine the growth response of *P. mamillatum* in pure culture towards these different carbohydrates showed that only xylose and pectin were not available to the fungus as a source of carbon. Thus the fact that xylose and pectin do not induce colonization by *P. mamillatum* in soil seems to depend on certain inherent physiological characteristics of the fungus. It might be difficult to reconcile this hypothesis with the fact that pectinase enzyme systems have been reported for some species of *Pythium* (Mishra, 1953; Ashour, 1954; Wood, 1955; Gupta, 1956), although *P. mamillatum* was not included in the reports of the investigations here cited.

Table 2. *Percentage colonization of wood fragments amended with carbohydrates*

	Natural inoculum				15 % inoculum			
	Replicate flasks				Replicate flasks			
	1	2	3	Mean	1	2	3	Mean
Water	0	0	4	1	47	38	45	43
Xylose	0	0	0	0	52	39	27	39
Glucose	52	40	19	37	78	60	50	63
Mannose	32	27	16	25	80	68	79	76
Maltose	20	28	40	31	79	87	60	75
Sucrose	36	14	16	22	32	78	68	57
Raffinose	30	19	18	22	70	85	74	76
Dextrin	44	14	43	34	66	94	86	82
Starch	32	70	23	42	56	76	85	73
Pectin	0	0	0	0	23	45	42	37

Nitrogen-containing substances

Wood fragments were autoclaved in 2 % (w/v) solutions of the following: potassium nitrate, ammonium chloride, glycine, glutamic acid, peptone, casein, albumen, haemoglobin and water control. Again, in the case of insoluble amendments, the presence of the material on or in the wood was deemed sufficient for the purpose of the experiment. The experimental arrangement was similar to that described above for carbohydrates. The percentage of fragments finally colonized by *P. mamillatum* is given in Table 3.

Amendment with organic nitrogenous substances increased the percentage of fragments finally colonized by *P. mamillatum* compared with controls receiving water only, this being effected by amino acids as well as by more complex proteinaceous compounds. The inorganic nitrogen

amendments, potassium nitrate and ammonium chloride, did not affect percentage colonization of wood fragments from the natural inoculum level of soil A, but at the 15 % inoculum level colonization was increased above that in the controls. The explanation of this behaviour might well lie with the fact that the two inoculum levels thus compared differ qualitatively as well as quantitatively. A high proportion of the fungal propagules in normal soil A, especially after drying, are probably resting propagules, whereas maizemeal-sand inoculum of the fungus contains, on the other hand, a higher proportion of actively growing hyphae than that in a 'resting' soil. At natural inoculum level, inorganic nitrogen in soil substrates did not induce colonization by *P. mamillatum*. The point is raised, therefore, as to whether organic nitrogenous substances promote substrate colonization because of the carbon-containing part of the molecule rather than because of the nitrogen component.

Table 3. *Percentage colonization of wood fragments amended with nitrogenous substances*

	Natural inoculum				15 % inoculum			
	Replicate flasks				Replicate flasks			
	1	2	3	Mean	1	2	3	Mean
Water	0	0	4	1	66	37	50	51
KNO_3	0	4	0	1	95	82	84	87
NH_4Cl	0	0	8	3	90	85	63	79
Glycine	14	33	54	34	90	96	95	94
Glutamic acid	29	22	6	18	73	95	73	80
Peptone	81	78	83	77	100	100	95	98
Casein	82	80	86	83	100	89	85	91
Albumen	64	35	85	61	100	100	100	100
Haemoglobin	33	38	33	35	100	100	100	100

Concentration of carbohydrate

Glucose was selected as a representative carbohydrate. Wood fragments were autoclaved in the following percentage concentrations of glucose: 0·5, 1, 2, 5, 10 and 0 (distilled water control). For each concentration nine flasks were prepared, three containing soil A only, three containing soil A + 10 % inoculum, and the remainder with soil A + 20 % inoculum. Twenty-five of the appropriately supplemented wood pieces were mixed with the soil in each flask, and the percentage colonized by *P. mamillatum* determined after 3 days (Table 4).

These results show that at each inoculum level there was a large increase in percentage of fragments colonized by *P. mamillatum* when the glucose concentration was increased from 0·5 to 1 % but, with further increase in glucose concentration to 2 %, there was only a small corresponding increase in percentage colonization. Finally, for concentrations of glucose above 2 %, percentage colonization decreased. Thus the concentration of simple sugars in a substrate in soil can be too high for good colonization by *P. mamillatum*. The possibility had been considered that increase in concentration of glucose in wood fragments might result in a corresponding increase in the number colonized by *P. mamillatum* until all the fragments

were recorded as colonized, the hypothesis being that the increased quantity of glucose in the wood might cause an increase in the volume of the stimulating diffusion zone around each fragment; or, assuming no such increase, the additional glucose might serve to make the existing zone more favourable to activity of *P. mamillatum*. It must also be recognized, however, that increase in glucose content of the wood substrate would similarly increase numbers and activity of other soil fungi and bacteria in the near vicinity. Antagonism from this source would be more intense at higher glucose concentrations and could therefore account for the low colonization observed under these conditions.

Table 4. *Percentage colonization of wood fragments amended with different concentrations of glucose*

	Natural inoculum				10% inoculum				20% inoculum			
	Replicate flasks				Replicate flasks				Replicate flasks			
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
Water	0	0	0	0	36	24	27	29	45	46	33	41
Glucose, 0·5%	15	11	9	12	48	51	65	54	53	54	50	52
1·0%	25	29	32	29	62	67	75	68	83	73	66	74
2·0%	32	30	33	32	71	71	63	68	78	76	81	78
5·0%	12	15	21	19	55	42	55	51	44	44	55	52
10·0%	9	0	10	6	43	33	40	39	35	40	45	40

Table 5. *Percentage colonization of wood fragments amended with glucose and nitrate to give different* C/N *ratios*

	% KNO_3							
	0				0·5			
	Replicate flasks				Replicate flasks			
Glucose (%)	1	2	3	Mean	1	2	3	Mean
0	9	8	4	7	5	0	0	2
0·5	37	27	32	32	32	36	36	35
1·0	62	56	59	59	66	60	62	63
2·0	62	71	67	67	73	74	74	74

	1·0				2·0			
	Replicate flasks				Replicate flasks			
Glucose (%)	1	2	3	Mean	1	2	3	Mean
0	0	0	0	0	4	0	0	1
0·5	40	31	43	38	26	38	32	32
1·0	48	52	50	50	48	45	52	48
2·0	71	67	65	67	58	71	64	64

Carbon/nitrogen ratio

Solutions of glucose and potassium nitrate at percentage concentrations of 0, 1, 2 and 4 were prepared and combined factorially, so that each concentration of the one chemical existed at each of the four concentrations of the other. This gave sixteen treatments in all, in which the final concentration of each component was in the series 0, 0·5, 1 and 2%. Seventy-five

fragments of wood were added to each of the sixteen prepared solutions and autoclaved, twenty-five being placed in each of three flasks containing 50 g. soil A at natural inoculum level. The percentage colonized by *P. mamillatum* was then assessed (Table 5).

These results show clearly that for any of the concentrations used, and in whatever combination with glucose, potassium nitrate had no effect in increasing, above the levels obtained in the controls, the number of fragments colonized. In fact, colonization tended to decrease slightly with increasing concentrations of nitrate up to 2 %, probably due to a slight toxic effect. Increase in concentration of glucose amendment up to 2 % resulted in a corresponding increase in the number of fragments colonized, a result in agreement with that obtained in the preceding experiment. There can be little doubt, then, that primary colonization of organic substrates by *P. mamillatum* is not affected by the ratio of carbon to nitrogen in the substrate, but is, in effect, a direct response to a carbon source.

pH *of the substrate*

Although the nutritional status of the substrate has been shown to have special importance for colonization by *P. mamillatum*, certain other conditions of the material might also be involved. In this respect, pH of the substrate seemed worthy of closer examination. Buffers of citric acid and of sodium phosphate were used to prepare 20 ml. aliquots of solution with the following pH values: 3·2, 4·2, 6·2 and 8·2. In each of these was dissolved 0·4 g. glucose, giving a final concentration of approximately 2 %. Wood fragments were autoclaved in water, and then 150 pieces were soaked in each of the glucose solutions at the various pH values for 24 hr. For each pH treatment six flasks were prepared, three with normal soil A, and three with soil A + 15 % (w/w) inoculum (Table 6).

Table 6. *Percentage colonization of wood fragments at different* pH *values*

	Natural inoculum				15 % inoculum			
	Replicate flasks				Replicate flasks			
pH	1	2	3	Mean	1	2	3	Mean
3·2	0	0	0	0	35	14	23	24
4·2	36	41	45	41	71	73	75	73
6·2	70	67	69	69	90	95	95	93
8·2	37	23	38	33	63	48	50	54

These results indicate that pH of the substrate had a marked effect on saprophytic colonization by *P. mamillatum*. At pH 3·2 colonization was very low indeed, and was completely inhibited from the natural inoculum. Best colonization was found where the wood fragments had been maintained at pH 6·2, whilst at pH 8·2 the alkaline conditions were somewhat inhibitory.

Effect of the soil environment

Considerable data have accumulated on the influence of soil environmental conditions on the disease cycle of *Pythium* species. It is not possible, however, to extrapolate these data accurately into the saprophytic phase, chiefly because of the influence of these factors on the host plant as well as on the behaviour of the fungus. In this section, the results of an investigation into the effects of environmental conditions on the saprophytic colonization of organic material by *P. mamillatum* are presented.

Soil-water content

Pieces of turnip, wood, and leaf were prepared and sterilized. Soil A, air-dried, was weighed out into twenty-seven 100 ml. flasks, and soil A with 15 % (w/w) inoculum of *P. mamillatum* was placed in a further twenty-seven flasks. Nine flasks with normal soil A, and nine flasks of soil A + inoculum, were made up to 20 % m.h.c. and a similar number were made up to 50 and to 80% m.h.c., respectively, by adding the required quantity of tap water to each flask. Twenty-five turnip pieces were then placed in each of three replicate flasks for each soil-moisture content, at both natural and added inoculum levels; leaf and wood fragments were distributed in a similar manner to other series of flasks. The percentage of fragments colonized by *P. mamillatum* was determined after 3 days (Table 7).

Table 7. *Percentage colonization of organic materials at different soil-moisture contents*

	Soil moisture content (% m.h.c.)	Natural inoculum				15 % inoculum			
		Replicate flasks				Replicate flasks			
		1	2	3	Mean	1	2	3	Mean
Turnip	20	40	47	40	42	100	72	82	84
	50	55	63	53	57	78	74	81	78
	80	76	84	62	74	90	92	100	94
Wood	20	0	0	0	0	8	25	0	11
	50	0	0	0	0	33	23	36	31
	80	0	0	0	0	44	41	45	43
Leaf	20	0	0	0	0	33	59	47	46
	50	65	47	52	54	87	90	88	88
	80	100	81	100	94	100	100	100	100

Saprophytic colonization of all three organic substrates was clearly affected by the water content of the soil. Colonization was, in general, lowest at 20, and highest at 80 % m.h.c. Wood fragments, containing relatively few components suitable for utilization by *P. mamillatum*, were not colonized at all by the natural inoculum of soil A, even though the water content was favourably increased. On the other hand, turnip showed considerable colonization under the adverse conditions of 20 % m.h.c., this reflecting the particular suitability of this substrate for *P. mamillatum*. The effect of increased inoculum potential was to increase generally the percentage of fragments colonized.

Soil temperature

Four temperatures were tested: 5, 10, 20 and 30° C. Eighteen flasks (nine with 50 g. soil A, and nine with 42·5 g. soil A) were incubated at each temperature for 24 hr. prior to setting up. After this, the flasks with 42·5 g. soil received 7·5 g. inoculum of *P. mamillatum* (i.e. 15 % w/w). Of the eighteen flasks per temperature treatment, three series of triplicate flasks containing normal soil A received pieces of turnip, wood, and leaf respectively (at the rate of twenty-five pieces to each of three flasks), and similarly for the series of flasks with added inoculum. The percentage of pieces colonized by *P. mamillatum* was then determined after a further 3 days in the incubators (Table 8).

Table 8. *Percentage colonization of organic materials at different soil temperatures*

		Natural inoculum				15 % inoculum			
		Replicate flasks				Replicate flasks			
	(° C.)	1	2	3	Mean	1	2	3	Mean
Turnip	5	14	0	17	11	91	100	100	97
	10	100	100	100	100	100	100	100	100
	20	62	61	75	66	100	100	100	100
	30	47	37	47	44	69	75	80	75
Wood	5	0	0	0	0	95	92	90	92
	10	9	0	0	3	100	100	100	100
	20	10	0	0	3	80	82	82	81
	30	0	0	0	0	21	27	13	20
Leaf	5	0	0	0	0	56	60	81	65
	10	0	0	0	0	95	100	95	97
	20	0	0	0	0	43	33	32	36
	30	0	0	0	0	14	19	10	14

The results show several points of interest. In the first place, saprophytic colonization by *P. mamillatum* was highest at 10° C. for all three organic materials, and tended to decrease at 20 and 30° C. This was in contrast to the behaviour of *P. mamillatum* in culture, where the optimum temperature for growth is 30° C. The decrease in colonization at the higher temperatures can best be explained on the basis of a microbiological factor. At 20 and 30° C., the indigenous flora is sufficiently active to limit severely the activities of *P. mamillatum*. At 10° C., however, the activity of the soil flora is much lower, and *P. mamillatum*, being active at this temperature, readily colonized the material. At 5° C., on the other hand, the activity of both *P. mamillatum* and the soil flora would have been reduced by the low temperature. Nevertheless, at 5° C. considerable colonization was found at the 15 % inoculum level. This anomaly is explained as a result of experimental technique. All treatments were allowed to reach equilibrium at the appropriate temperature for 24 hr., and it was not until then that active inoculum (at a higher temperature) was placed in a situation of comparatively low microbiological activity, so that, before succumbing to the effect of the low temperature in this treatment, it was able to effect considerable colonization.

Soil pH *value*

The pH of soil A, normally 5·4, was modified to pH 4·2 and to pH 7·0 by the use of N/20 sulphuric acid and of calcium hydroxide, respectively. The soil was then allowed to equilibrate over a period of 2 months, and finally air-dried for experimental use. For each pH, nine flasks of natural soil A and nine flasks with soil + 15 % inoculum were prepared. Twenty-five sterilized fragments of turnip, wood and leaf were added to triplicate flasks in each of these groups. Colonization was assessed after 3 days (Table 9).

These results indicate the considerable influence of soil pH upon saprophytic colonization by *P. mamillatum*. Lowering the pH value of soil A to 4·2 resulted in a decrease in colonization, whereas at pH 7·0 colonization was generally increased above that at pH 5·4.

Table 9. *Percentage colonization of organic materials at different soil* pH *values*

		Natural inoculum				15 % inoculum			
		Replicate flasks				Replicate flasks			
	pH	1	2	3	Mean	1	2	3	Mean
Turnip	4·2	22	7	29	19	69	65	76	70
	5·4	64	59	47	53	100	90	100	97
	7·0	50	63	56	56	90	89	89	89
Wood	4·2	0	0	0	0	53	65	44	54
	5·4	0	0	0	0	75	71	81	76
	7·0	19	20	10	16	84	84	72	80
Leaf	4·2	0	5	0	2	56	77	78	70
	5·4	0	0	0	0	100	100	100	100
	7·0	56	72	66	65	100	100	100	100

Soil texture

Soil texture has been reported to influence *Pythium* activity. Remy (1949) considered that clay soils contained greater quantities of *Pythium*, and Vámos (1954) showed that damping-off of pine seedlings was more prevalent in clay soils. In view of this, it seemed desirable to investigate saprophytic colonization by *P. mamillatum* under clay as compared with sandy soil conditions. Dried soil A was mixed in bulk with 25 % (w/w) washed quartz sand, on the one hand, and with 25 % (w/w) clay, on the other. After remoistening, and incubating for 1 month, the soils were air-dried before use. The m.h.c. of the modified samples was determined by the method of Keen & Raczkowski (1921). The m.h.c. of soil A plus clay was 80 ml./100 g. air-dry soil, and that of soil A + sand was 45, as compared with a value of 48 for normal soil A. All soil samples were made up to 50 % m.h.c. before use. For each soil treatment, nine flasks of soil without artificial inoculum were prepared, and nine flasks of soil + 15 % inoculum of *P. mamillatum*. Twenty-five pieces of turnip, wood and leaf were added to triplicate flasks in each group of nine, and colonization by *P. mamillatum* determined after 3 days (Table 10).

It is apparent from these data that colonization was greatest in the

clay-soil treatment, this being especially evident from the series at natural inoculum level. Addition of sand to soil A, on the other hand, did not seem to have exercised a significant effect upon percentage saprophytic colonization by *P. mamillatum*. Since normal soil A itself is largely composed of sand, it is evident that 25 % additional sand might be incorporated into the physical structure without much affecting physical properties of the soil (see m.h.c. values). It is difficult to define precisely the changes induced in a soil when the texture is altered by the addition of clay, but, with *P. mamillatum* in the present instance, water availability is likely to have been the chief factor concerned in the observed increase of activity by this fungus.

Table 10. *Percentage colonization of organic materials in soils of different texture*

		Natural inoculum				15% inoculum			
		Replicate flasks				Replicate flasks			
	Addition to soil	1	2	3	Mean	1	2	3	Mean
Turnip	Clay	100	94	83	92	82	94	100	92
	None	61	94	65	73	70	92	90	84
	Sand	48	68	62	59	75	86	76	79
Wood	Clay	0	27	0	9	100	67	82	83
	None	0	0	0	0	59	50	46	52
	Sand	0	0	0	0	58	48	73	59
Leaf	Clay	83	64	93	80	82	90	83	85
	None	14	13	38	22	69	72	70	70
	Sand	13	0	0	4	80	77	92	83

Discussion

Colonization of a substrate by a fungus might be expected to be related to the presence of nutrients available, under the given conditions, to that fungus. Since *P. mamillatum* is recognized as a colonizer of virgin organic material, there should be some correspondence between the sort of materials stimulatory to colonization of substrates in soil, and the materials that the fungus can utilize in pure culture. This is here shown to be true, especially in respect of nutrients providing a carbon source.

The chief conclusion to be drawn from this study of the effect of environmental factors on saprophytic colonization by *P. mamillatum* is that soil pH, water content, and texture affect this activity largely by direct action on the fungus itself. On the other hand, the influence of soil temperature provides an excellent example of how the saprophytic activity of a fungus in soil may be so curtailed by competition from other organisms that the optimum temperature for saprophytic colonization is far removed from the growth optimum exhibited in pure culture. By inference then, soil temperature would seem to affect the activity of the indigenous soil flora more than do the other environmental factors here considered.

The two environmental factors which fluctuate seasonally in any particular soil are temperature and water content, and these factors would thus, through their effects on saprophytic activity, play some part in determining seasonal parasitic potential of *Pythium* in soil. Saprophytic activity

was greatest in soil at 10° C. and at high water content, conditions more typical of spring than of any other season. This agrees with the conclusions reached by McLaughlin (1947), who put forward evidence for a peak of pathogenic activity by *Pythium* in soil during spring.

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AUTORADIOGRAPHY OF WATER-SOLUBLE COMPOUNDS IN FUNGI

By JUNE ROBSON

Isotope Research Division, Atomic Energy Research Establishment, Harwell

(With Plate 19)

A method for locating water-soluble radioactive materials in fungi by freeze drying them, sectioning in paraffin wax and applying A.R. 10 stripping film in chloroform is described.

Holt, Cowing & Warren (1949) and Holt & Warren (1952, 1953) have described methods for the gross autoradiography of animal tissues containing water-soluble radio-active compounds, and Canny (1955) gives a method for the high resolution autoradiography of soluble substances in algae. None of the techniques can be directly applied to the study of cellular distribution of water-soluble radio-active substances in fungi. Holt & Warren's technique of applying film to the specimen does not give the good resolution (1–2 μ) of which the ^{35}S is capable. Canny's technique of spraying algal cells onto a slide and freeze drying them *in situ* would break the fungal cells and so is unsuitable. Therefore, a technique combining the freeze drying of tissue due to Holt & Warren with Canny's method of applying stripping film to the specimen has been used for making autoradiographs of fungi containing water-soluble compounds.

Mycelium grown in a fluid medium is drained to remove excess liquid and placed in a centrifuge tube. As fungal tissues adhere firmly to the glass container when they are freeze dried in the presence of culture fluid, the tube is lined with a coating made by shaping strips of Parawax (Gallenkamp and Co. Ltd.) round a rod of suitable dimensions, and heat sealing the edges. The specimen is then frozen on an Edwards Centrifugal Freeze-drier (Model LB.5) attached to a vacuum line, pumped and dried for 4–5 hr. The specimen, thoroughly dried, is embedded in wax under vacuum as follows. The Parawax bag containing the fungus is pinned to the centre of a block of embedding wax in a small beaker. This is placed in a vacuum-drying oven containing silica gel, and dried at room temperature overnight. The temperature of the oven is then raised to 60° C., the Parawax bag melts and the fungus drops into the molten wax. Should a clearing agent be required, a little can be poured on to the solid wax before the specimen is put in the oven. The fungus is mounted in wax blocks and sections *c.* 3 μ cut on a Cambridge Flat-cutting Microtome. The thickness indicator was set at 2 μ, so that all the sections, allowing for any inaccuracy in the microtome, would be within the 5 μ limit for high-resolution autoradiography (Boyd, 1955, p. 113). The sections are dewaxed with xylol, and kept in a dry-sealed staining dish until A.R. 10 stripping film (Kodak Ltd.) is applied to them. Canny's method (1955) was used to apply the

film to the slide. A piece of plate glass $2\frac{1}{2} \times 3\frac{1}{2}$ in. was used to support the film whilst it was washed with chloroform, and the film was transferred to the slide in a bath of chloroform. Films are exposed at 0° C. in a sealed air-tight container, and later developed normally.

Spores can be treated in the same way as hyphal material. Since it is easier to examine them later in the mass rather than dispersed in the wax, the centrifuge tube is not initially lined. When the clump of spores has been freeze dried, the tube is removed from the drier and hot wax poured into it; the spores do not disperse because they adhere to the glass. The tube is left in the embedding oven for 1 hr. so that the wax penetrates the spores completely and, provided the wax is cooled rapidly and without agitation, the spore mass remains intact.

The block of spores is removed by carefully breaking the glass. Should it be necessary to section the spores to discover the internal distribution of metabolites, they can be treated in the same way as hyphae. When the exact position of the metabolite is not important, the block can be sectioned with a razor blade, since many fungus spores are small enough to be autoradiographed intact.

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EXPLANATION OF PLATE 19

Fig. 1. Spores of *Penicillium chrysogenum*, 49.133, placed in a medium containing ^{35}S for 2 hr., showing radio-activity around the spores. × 3000.

Fig. 2. Freeze-dried preparation identical with that shown in fig. 1, except that spores were washed with water before applying stripping film. × 3000.

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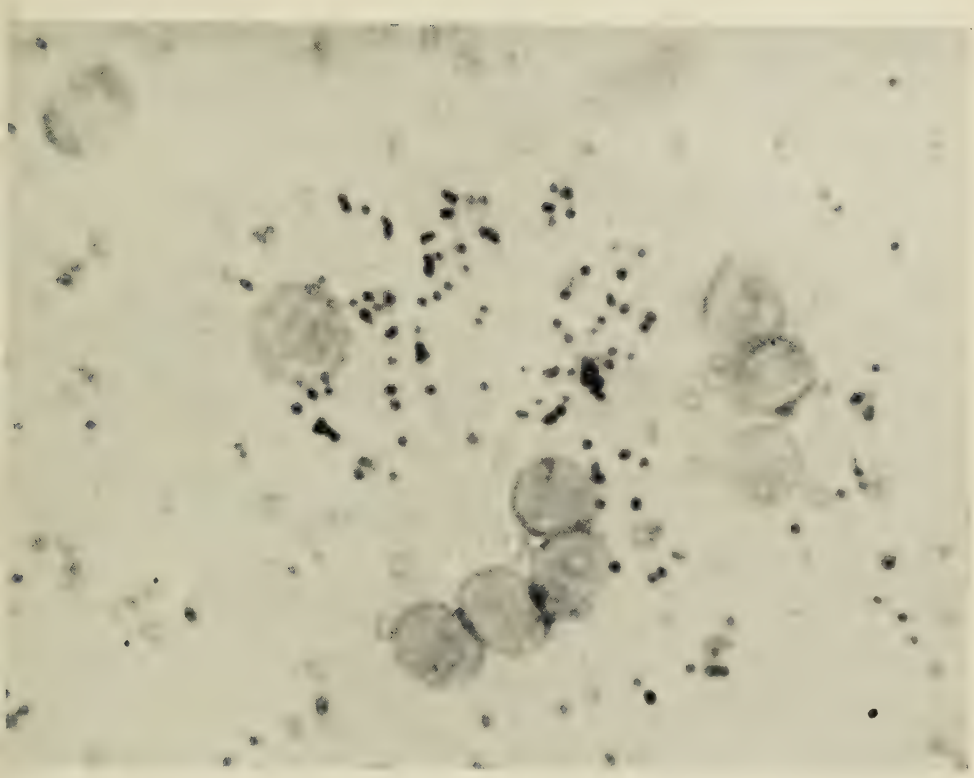

Fig. 1

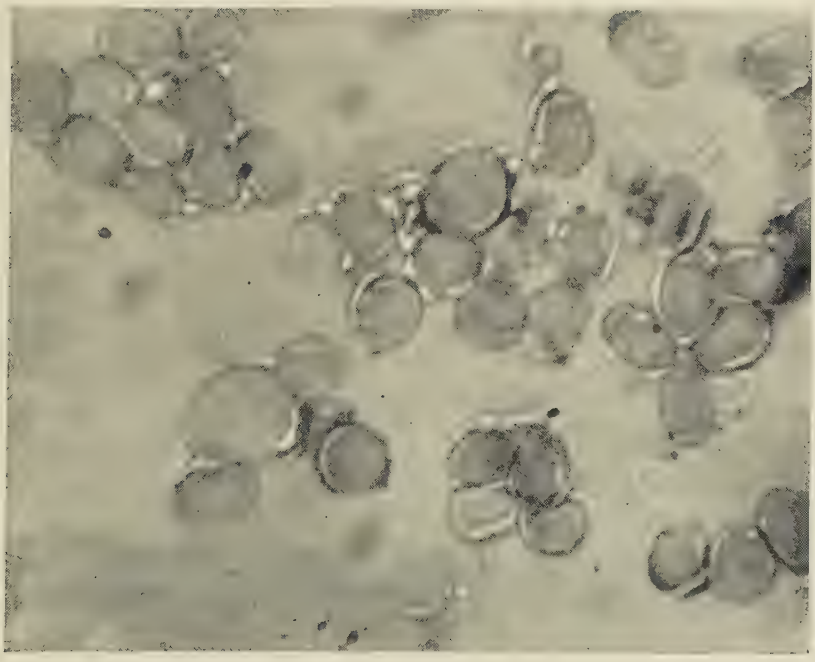

Fig. 2

(*Facing p.* 542)

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ADVANCES IN THE SEROLOGY OF FUNGI*

By H. P. R. SEELIGER

Hygiene-Institut der Rheinischen Friedrich-Wilhelms-Universität Bonn/Rh.

(With Plate 20)

The serological methods applicable to fungi are briefly reviewed, and their uses are critically discussed. Serological studies on the black yeasts and on other yeasts are used to illustrate the applications of serology in the elucidation of taxonomic relationships in fungi.

Although serological test procedures have been applied in the mycological field since early this century, only in recent years have some of them come into general use. About two years ago, after doing some work of my own, I tried to collect together all the widely scattered literature on fungus serology which covers a very wide field of experimental and clinical research, with a still increasing number of test procedures (Seeliger, 1958). At that time some 500 papers had already been published. The recent interest in the application of serological methods to the study of fungi and mycotic diseases may be exemplified by the fact that within two years more than fifty new important contributions from all parts of the world have dealt with fungus serology. This seems to be compatible with the assumption that some serological tests have already become firmly established as useful and reliable in the identification of certain groups of fungi and in the study of several mycotic infections.

Generally speaking one may say that all serological test procedures which have so far been applied in mycology have been derived from similar or identical methods used in bacteriology. These are summarized in Table 1.

The mycologist, however, has to cope with many more technical difficulties than the bacteriologist. The primary reason for this is that the cultural properties of many fungi are quite different from those of bacteria. Thus special procedures have to be devised before serological tests can be applied.

This holds true not only for the preparation of homogenized antigens to be used for agglutination and agglutinin-absorption studies but also for the production of suitable immune sera. In the preparation of antigens such methods as continuous shaking of cultures, sonic oscillation, mechanical grinding and homogenization have been of great value. It was found that the immunizing capacity of fungal antigens shows extreme variations. With some fungi, e.g. species of *Cryptococcus* and other capsulated and non-capsulated yeasts, no reliable method of immunization has as yet been

* Based on a lecture at a meeting of medical and veterinary mycologists arranged by the Medical Mycology Committee of the Medical Research Council, Bristol, April 1959.

worked out. The respective results of many investigators show considerable differences.

Three procedures of preparing fungal antisera have mainly been used, the third only recently: (i) intravenous injection of live or killed homogenized antigens or soluble substances; (ii) infection of animals with pathogenic fungi; and (iii) subcutaneous injection of homogenized antigens together with Freund's adjuvants. Freund's adjuvants (Freund, 1956) consist of mixtures of killed tubercle bacilli with emulsions of paraffin (Bayol F) and oleic acid compounds (Arlacel).

Although our experience is limited so far to the first two procedures, according to Pepys (1959, personal communication) it seems that the third route of immunization will eventually be the method of choice. If an animal is inoculated with fungal antigens, serum antibodies may appear in significant titres within 2–4 weeks. In such instances a comparison of serum specimens taken before and after immunization shows that the antibody is mainly present in the gamma-globulin fraction from which it can be absorbed. Such an antiserum, the antibody of which can be absorbed by the homologous antigen, is useful for all kinds of serological test procedures.

Table 1. *Serological procedures applicable to the study of fungi and mycoses (skin tests not included)**

Reaction	Antigen
Agglutination reaction	Whole cells. Sensitized blood corpuscles. Sensitized particles (collodion, latex, resins etc.)
Capsular reaction	Whole cells
Precipitin test (classical method agar-gel-diffusion test. Immunoelectrophoresis)	Culture filtrates. Cell extracts. Chemical fractions of extracts (polysaccharides, proteins, etc.). Lysates
Complement-fixation test	Whole cells. Cell homogenates. Culture filtrates. Cell extracts. Lysates. Chemical fractions of soluble substances
Agglutinin-absorption test	Whole cells. Soluble antigens
Determination of fungicidal power in patient's serum	Living cells

* A detailed description of methodology is to be presented by the author in a new edition of *Handbuch für Haut- und Geschlechtskrankheiten* (in press).

In other instances, however, similar experiments may result in complete failure. Despite various methods of immunization and intensive treatment of the animals with antigens, no antibodies may become demonstrable, even after a long-term immunization. This is almost the rule with capsulated yeasts, but such failures have also been encountered with other fungi which do not possess a trace of a capsule. Some investigators ascribe this to a phenomenon identical with or related to the so-called 'immunological paralysis' in which antibodies are produced, but they escape detection because of their immediate absorption by antigens bound to the tissues. This absorption is followed by the destruction of the antigen-antibody complex. This difficulty may be overcome by a short-term immunization procedure using relatively low dosages of antigen. Successful results were reported by Neill, Castillo, Smith & Kapros (1949).

Specificity of reaction

As in other fields of microbiology, serological reactions with fungal antigens and antisera are not absolutely specific. Cross-reactions are quite frequent, particularly between species of one genus or between related fungi. The degree of serological specificity in a given antiserum depends very much on the length of the immunization procedure. The longer it is, the more overlapping antibodies may become demonstrable. By proper dilution of the antiserum or by Castellani's absorption test as applied by Almon & Stovall (1934), Benham (1937), Evans (1950), Kaufmann (1944–45), Jonsen (1955), Martin (1942), Nordén (1951), Seeliger (1954, 1957, 1958, 1959) and others, such cross-reactions can be eliminated to some extent.

In some genera the antigenic mosaic is complex. Then antigenic analysis can be performed only by use of a set of different *factor sera*, which are prepared by absorption of antisera with antigens of heterologous fungi. This was tried by many investigators (for references see Kligman & DeLamater, 1950; Seeliger, 1958). The value of this procedure has conclusively been demonstrated in Japan by Tsuchiya and his collaborators (1957, 1958, 1959) during a long series of experiments involving a great number of fungus species. Thus it was possible to work out antigenic schemes for yeasts of the genera *Candida*, *Hansenula*, *Saccharomyces*, etc. In our own experiments (Seeliger, 1957) we were able to recognize several serogroups in *Rhodotorula* and *Sporobolomyces* (Table 2). During these experiments standard cultures of all species recognized by Lodder & Kreger-Van Rij (1952) and freshly isolated strains of these two genera were used. It may be of interest to the taxonomist that no major antigenic communities could be detected between *Rhodotorula* and *Sporobolomyces*, although several species of both genera have many cultural and biochemical features in common. Agglutination and precipitation studies gave identical results. Owing to difficulties in obtaining homogeneous suspensions of some *Sporobolomyces* strains, precipitin tests proved superior (Results, Table 2). Tsuchiya *et al.* (1957) likewise have presented an antigenic scheme of the *Rhodotorula* group. For *R. mucilaginosa*, *R. rubra*, *R. minuta* and *R. pallida* the Japanese and German investigators obtained very similar results.

One must, however, be very cautious in the interpretation of results of agglutination reactions, even with monovalent factor sera. Serological identity of two different fungus species cannot be based on agglutination tests alone, but must be confirmed by cross-absorption studies with whole antigens and non-absorbed antisera. It should be remembered that the serological behaviour of any bacterial or fungal antigen depends on (*a*) its antigenic power, (*b*) its agglutinating or precipitating capacity, and (*c*) its antibody-binding properties.

With this in mind one can hope that the recent advances in yeast serology will give a sound basis for future work.

Antigenic analysis has proved an interesting tool in the demonstration of the identity of fungus species assigned to different genera or even families, according to the presence or absence ofascospore-formation. Thus it was confirmed serologically that *Candida pseudotropicalis* and *Saccharomyces*

fragilis possess the same antigens, likewise *Candida mycoderma* and *Pichia membranefaciens* (Seeliger, 1958). *Allescheria boydii* and its imperfect state *Monosporium apiospermum* are serologically identical (Seeliger, 1954, 1956).

Sometimes serological reactions with fungus antigens have been called 'non-specific', particularly in the study of fungal diseases of man. For instance, a positive histoplasmin skin test was described in a patient suffering from cryptococcosis. From such and similar observations one may gain the impression that serological reactions are not specific. Is this justified? From experimental studies it is known that antigens of *Histoplasma capsulatum* and *Cryptococcus neoformans* do not cross-react. It is, however, quite possible that a patient suffering from a mycotic infection, e.g. cryptococcosis, may have acquired a persisting histoplasmin hypersensitivity many years before the onset of his present disease. This means that serological reactions are specific with regard to the antigen, but they need not necessarily be specific for a given disease. This applies particularly for the presence of antibodies against *Candida albicans*. Owing to the wide spread of the organism among the human population, a high number of positive reactors are usually found in skin testing and serum studies. This, of course, makes the interpretation of positive findings very difficult, if the attempt is made to use such procedures for diagnosis (Kligman & De-Lamater, 1950; Janke, 1955; Seeliger, 1954, 1958; Winner, 1955).

Let me stress again: there is *no such thing as absolute antigenic specificity*. The number of antigens or antigenic constituents in nature is limited. Therefore it is not surprising to find serological cross-reactions even between antigens of rather different origin. To give a few examples from many others: antisera of cryptococci give cross-reactions with certain pneumococcal serotypes (Rebers, Barker, Heidelberger, Dische & Evans, 1958) and with other substances of high molecular weight (Evans, Sorensen & Walls, 1953). Similarly, antisera against *Sporotrichum schenckii* will precipitate polysaccharides of some pneumococci (Neill, Castillo & Pinkes, 1955). There are even common antigenic fractions between *Candida albicans* and salmonellae of o-group C (Aksoycan & Kauffmann, 1957). This reminds one of the antigenic relationship between human blood cells and certain pollen extracts. From such findings, no one would doubt serological specificity as it exists in practice.

Serologically the polysaccharide fractions of the antigens function as haptenes and determine the degree of the specificity. Protein constituents and lipids tend to provoke reactions with decreased specificity.

I should now like to present a few examples of the possibilities and limitations in the application of fungus serology.

Some applications

They concern observations on the antigenic relationships among the dimorphic, black pigment producing, *yeast-like* fungi which, because of their morphological and cultural properties, present rather difficult problems to the mycologist and particularly to the taxonomist. Here I shall give a little more detail.

Table 2. *Results of precipitin tests with polysaccharide extracts of species of* Rhodotorula *and* Sporobolomyces

(After Seeliger, 1957, simplified)

Antigen	Antiserum* 1	2	3	4	5	6	7	8	9	10	11–19
1. *R. mucilaginosa*	+++	+++	–	(±)	(±)	(±)	–	–	–	–	–
2. *R. rubra*	++	+++	–	(+)	–	(±)	–	–	–	–	–
3. *R. glutinis* var. *satoi*	–	++	(±)	++	+	–	–	–	–	–	–
4. *R. glutinis* var. *rubescens*	–	++	–	–	+	–	–	–	–	–	–
5. *R. glutinis*	–	–	–	(±)	+	–	–	–	–	–	–
6. *R. minuta*	–	–	–	–	–	+++	+++	+++	–	–	–
7. *R. texensis*	–	–	–	–	–	+++	+++	+++	–	–	–
8. *R. pallida*	–	–	–	–	–	+++	+++	+++	–	–	–
9. *R. aurantiaca*	–	–	–	–	–	–	–	–	++	–	–
10. *R. flava*	–	–	–	–	–	–	–	–	–	+	–
	11	12	13	14	15	16	17	18	19		1–10
11. *S. odorus*	+++	++	+	–	±	–	–	–	–		–
12. *S. holsaticus*	+++	++	–	–	±	–	–	–	–		–
13. *S. salmonicolor*	+++	±	+++	+	+	–	–	–	–		–
14. *S. roseus* var. *salmoneus*	+++	–	++	+++	+++	++	+	–	–		–
15. *S. pararoseus*	+	–	–	+++	+++	++	+++	–	–		–
16. *S. roseus* var. *madurae*	±	–	–	++	++	++	+++	–	–		–
17. *S. roseus*	±	–	–	+	+	+	+	–	–		–
18. *S. gracilis*	±	–	–	±	±	–	–	+	–		–
19. *S. albo-rubescens*	–	–	–	–	–	–	–	–	+		–

* Number corresponding to the number of the homologous antigen.

Serology of the black yeasts

The first serological studies on black yeasts were reported in England by Stone (1930), who successfully applied the complement-fixation test. Later Conant & Martin (1937) again used this reaction in the study of the causal agents of chromoblastomycosis. These studies were extended by Trejos and Seeliger (see Seeliger, 1957, 1958), who employed the agglutination test with formolized suspensions of the yeast phases of various dematiaceous fungi. In addition, precipitin studies were performed with partially purified polysaccharide extracts from test cultures.

The specificity of the agglutination reactions with homologous and heterologous rabbit antisera obtained after intensive immunization with formolized broth cultures is demonstrated in Pl. 20, fig. 1, which shows yeast cells of *Pullularia bergeri* (syn. *Torula bergeri*) in the presence of 1 : 10 diluted antiserum against *P. werneckii* (syn. *Cladosporium werneckii*), no reaction being visible; and in Pl. 20, fig. 2, where the same antigen suspension is seen after mixing with homologous antiserum likewise diluted 1 : 10. Immediately a strong agglutination reaction occurred. Such reactions take place on the slide as well as in the test tube and are clearly observable with the naked eye. They can be differentiated from spontaneous clumping sometimes present in yeast suspensions. In a similar way the precipitin tests give clear-cut reactions.

Table 3 gives the results of tube-agglutination tests with strains of three *Pullularia* and two *Sporotrichum* species. Besides species-specific reactions, one can observe some serological overlapping, which apparently is due to group or genus-specific factors of the antigens.

Table 3. *Agglutination reactions with black yeast-like fungi*

	Antiserum				
Antigen	*P. pullulans*	*P. bergeri*	*P. werneckii*	*S. gougerotii*	*S. schenckii*
P. pullulans	320*	40	40	—†	—
P. bergeri	40	2560	—	—	—
P. werneckii	40	—	160	—	—
S. gougerotii	—	—	—	640	—
S. schenckii	—	—	—	—	640

* Reciprocal serum titre. † Negative at 1 : 20.

From Table 4 it will be seen that the precipitin reactions with partially purified carbohydrate extracts give quite similar results (as in the agglutination tests).

From the outcome of these investigations extended by the study of a collection of cultures from Prof. Lacaz, São Paulo, a tentative antigenic scheme was worked out based on the serological behaviour of fifty-six strains.

It was of considerable interest to examine whether *Pullularia werneckii* might be related serologically to *Cladosporium mansonii*, as both organisms have been described as cause of Tinea nigra. An antiserum prepared from Castellani's original strain of *C. mansonii* precipitated homologous extracts but also gave group reactions with extracts from *Phialophora verrucosa*,

Pullularia schawii and *Margarinomyces heteromorphus*. Such group reactions were not apparent with antigens from *Pullularia pullulans*, *P. bergeri* and *P. werneckii*.

There was no serological evidence that *Cladosporium mansonii* and *Pullularia werneckii* possess common antigenic factors. Antigenic relationship, however, was demonstrated—as stated already—between *Cladosporium mansonii* antiserum and two strains of *Margarinomyces heteromorphus*, one culture isolated by myself and identified by Dr De Vries, and the other obtained as reference strain from the Centraal Bureau voor Schimmelcultures, Baarn, Holland. With antigens and antisera from those three strains typical cross-precipitin reactions were observed. Whether this has any taxonomic or phylogenetic significance, remains to be seen.

Table 4. *Precipitation reactions with black yeast-like fungi*

Antigen	Antiserum: *P. pullulans*	*P. bergeri*	*P. werneckii*	*S. gougerotii*	*S. schenckii*
P. pullulans	+++	±	±	±	−
P. bergeri	+++	+++	±	±	−
P. werneckii	+	−	+++	−	−
S. gougerotii	−	−	−	+++	−
S. schenckii	−	−	−	−	+++
Phialophora jeanselmei	−	−	−	+++	−
P. verrucosa, P. compacta, P. lignicola, Cladosporium pedrosoi, C. trichoides, C. carrionii	−	−	−	−	−

Table 5. *Antigenic scheme of some yeast-like dematiaceous fungi*

(After Trejos & Seeliger, 1957.)

Species	Antigenic factors						
Pullularia pullulans	A	B	C	.	.	.	.
P. bergeri	.	B	.	D	.	.	.
P. werneckii	.	.	C	.	E	.	.
Sporotrichum gougerotii	.	.	.	.	.	F	.
Phialophora jeanselmei	.	.	.	.	.	F	.
S. schenckii	.	.	.	.	.	.	G

Moreover, antisera of both of these species agglutinated strongly, but in low dilutions only, a yeast-phase suspension of a strain of *Pullularia schawii*, which was identified by Lacaz and Ulson. Antigens of this particular strain did not react with our antisera against the other recognized species of *Pullularia*. Unfortunately our attempts to prepare a good antiserum from *P. schawii* have not been successful so far. But there is enough evidence that this species may be separated serologically from *P. pullulans*, *P. werneckii* and *P. bergeri*. The yeast phase of *P. schawii* was furthermore agglutinated very distinctly by antiserum from the so-called *Sporotrichum gougerotii*.

These findings may have some importance for the taxonomy of certain morphologically similar black yeasts. It should be remembered that by means of complement-fixation tests Stone (1930) first found that *S. gougerotii* and *Phialophora jeanselmei* could not be separated serologically. This also was borne out by Seeliger and Trejos in 1955 and Seeliger *et al.* in 1958 by use of precipitin tests. In the course of these experiments this sero group

could be differentiated distinctly from *Pullularia bergeri*, previously called *Torula bergeri*. This is in partial contrast to the assumption of Trejos that culturally and morphologically *P. bergeri* belongs to *Phialophora jeanselmei*. The results with strains obtained from the collection of Prof. Lacaz and Dr Ulson, São Paulo, again demonstrated that smooth and rough strains, both designated as *P. jeanselmei*, are apparently neither identical with each other nor offsprings of each other. They are antigenically different and therefore in all probability members of two different species, only the smooth form of which is synonymous with *Pullularia bergeri*.

It has also been shown by several investigators (Nordén, 1951; Lurie, 1948; Seeliger, 1958) that *Sporotrichum schenckii* is serologically identical with its white variety, *S. beurmanii*. There can be no doubt that *S. schenckii* strains from all parts of the world represent one homogeneous sero group.

Precipitation reactions

The results of tube precipitation reactions were in line with findings from the application of the agar-gel precipitin test (Ouchterlony, 1948; Oudin, 1952; Seeliger, 1954, 1955, 1958; Kaden, 1957; Heiner, 1958; Pepys, 1959), which we have applied since 1953 in antigenic studies of fungi and which serves very well in serological studies of fungal diseases (Heiner, 1958). The complex nature of the antigens is revealed by a number of precipitation bands developing at the sites of the gel where soluble antigens react with the antibodies in optimal proportions. In the case of antigenic identity these lines tend to form *arcades* (*coalescence-phenomenon*). If only some of several precipitation bands meet each other or cross each other, this is interpreted as an indication of antigenic dissimilarity or common partial antigens.

In preliminary experiments with some aspergilli (Seeliger, 1958) it was found that some of the species are connected by group antigens which are demonstrable besides species-specific factors. Pepys (1959) and co-workers have used this method successfully in their studies on species of *Aspergillus* and *Cladosporium*. The same applies to our investigations on *Sporobolomyces* (Seeliger, 1957) and *Madurella grisea* (Seeliger, 1956). With several fungus extracts we have obtained as many as six precipitation bands.

This method seems well suited for the exploration of the antigenic material present in *skin testing vaccines* and even more in the *analysis of animal and human sera* taken from patients with fungus infections (Heiner, 1958) or fungus allergy (Pepys, 1959).

In our opinion, *agglutination tests* offer the greatest help in the study of fungi from which homogeneous antigens can be obtained. With conidial suspensions of hyphomycetes this sometimes requires the use of surface-active agents. In the preparation of *Nocardia* antigens, ultrasonic treatment has been found a promising method.

While agglutination reactions are restricted to certain groups of fungi, *precipitin* tests may be performed with practically all kinds of fungi. Investigations of the *Trichosporon* group have given some interesting results, e.g. serological evidence was obtained that some of the species are related to *Cryptococcus*, others to *Saccharomyces* and *Candida*, and again others to *Geo-*

trichum. In the same way the form-genus *Candida* could be subdivided. *C. curvata* and *C. humicola* were found to possess group antigens which are present only in *Cryptococcus* and certain members of *Trichosporon*.

With capsulated *Cryptococcus* strains, *capsular reactions* have permitted the establishment of at least three antigenic capsule types of *C. neoformans*, according to Evans (1950) and Evans & Kessel (1951). These capsular reactions are not caused by a swelling, but by a precipitation of the antibody on the surface of the capsules (Evans, Seeliger, Kornfeld & Garcia, 1956). With antisera obtained through the courtesy of Dr Evans and our own material we found, however, that non-pathogenic cryptococci likewise reacted with capsular antisera. This is demonstrated in Table 7.

Table 6. *Precipitin tests of various* Candida *and* Trichosporon *species and other yeasts with fungal antisera*

	Antiserum			
Antigen from	*C. albicans* = *S. cerevisiae*	*C. pseudotropicalis* = *S. fragilis*	*Cryptococcus laurentii* = *C. neoformans*	*G. candidum* = *N. elongata* = *E. magnusii*
Candida albicans	+++	–	–	–
C. tropicalis	+++	–	–	–
C. parapsilosis	++	–	–	–
C. mycoderma	+++	–	–	–
C. guilliermondii	++	–	–	–
Trichosporon behrendii	+++	–	–	–
Saccharomyces cerevisiae	+++	–	–	–
C. pseudotropicalis	–	+++	–	–
S. fragilis	–	+++	–	–
C. curvata	–	–	+++	–
C. humicola	–	–	+++	–
T. cutaneum	–	–	+++	–
T. infestans	–	–	+++	–
Cryptococcus laurentii	–	–	+++	–
C. neoformans	–	–	+++	–
Candida lipolytica	–	–	–	+++
C. rugosa	–	–	–	+++
T. capitatum	–	–	–	++
T. sericeum	–	–	–	+++
T. fermentans	–	–	–	++
Schizosaccharomyces pombe	–	–	–	+++
Geotrichum candidum	–	–	–	+++
Nadsonia elongata	–	–	–	+++
N. fulvescens	–	–	–	+++
Endomyces magnusii	–	–	–	+++
*C. krusei**	–	–	–	–
*C. utilis**	–	–	–	

* Strong reaction in homologous antiserum; *C. krusei* is serologically similar to *Pichia fermentans*.

These findings clearly show the limitation of fungus serology in this particular field. At the present time it is not possible to separate with certainty pathogenic from non-pathogenic cryptococci by use of agglutination, precipitation or capsular reactions, at least with the sera available. This is in line with observations on non-pathogenic cryptococci by Kaufmann (1944/45). But one is well able to separate serologically *Cryptococcus* from *Lipomyces*, which Benham (1955) has suggested is the perfect state of the former (see Table 7).

Table 7. *Agglutination and capsular reactions of* Cryptococcus, Torulopsis *and* Lipomyces *species in homologous and heterologous antisera*

(After Seeliger, 1958, 1959.)

	Antiserum (1:10 diluted)											
	C. neoformans											
		Evans-type										
	Polyvalent	A	B	C	*C. diffluens**	*C. luteolus*	*C. laurentii*	*C. terreus*	*Candida curvata*	*Candida albicans*	*Geotrichum candidum*	*Lipomyces lipoferus, L. starkeyi*
C. neoformans A (7)†	CA‡	CA	CA	–	–	–	–	–	A	–	–	–
C. neoformans B (1)	CA	–	CA	–	–	–	–	–	a	–	–	–
C. neoformans C (1)	CA	–	–	CA	–	–	–	–	a	–	–	–
C. diffluens (1)	CA	CA	CA	–	–	–	–	–	–	–	–	–
C. diffluens (2)	A	A	a	–	A	–	a	–	CA	–	–	–
C. diffluens (5)	A	a	–	–	a	–	a	–	a	–	–	–
C. diffluens (1)	a	–	–	–	–	–	–	–	–	–	–	–
C. albicans (2)	CA	CA	CA	–	–	–	a	–	CA	–	–	–
C. albicans (1)	A	A	a	–	–	–	a	–	A	–	–	–
C. albicans (1)	–	–	–	–	–	–	–	–	–	–	–	–
C. laurentii (1)	a	–	–	–	–	–	A	–	A	–	–	–
C. luteolus (1)	CA	CA	–	–	–	C§	–	–	CA	–	–	–
C. terreus (1)	–	–	–	–	–	–	–	A	(a)	–	–	–
T. aeria (1)	A	–	–	A	–	–	–	–	–	–	–	–
L. lipoferus/L. starkeyi (10)	–	–	–	–	–	–	–	–	–	–	–	CA or A

* Weak antiserum with low titre.
† Number of strains examined.
‡ A = strong agglutination, a = weak agglutination, C = capsular reaction.
§ No agglutination in repeated tube and slide tests.

Complement fixation

We have also used extensively this test, which is very sensitive and reveals many cross-reactions. It has, however, given promising results in the study of deep-seated mycotic infections, such as histoplasmosis, North and South American blastomycosis and coccidioidomycosis (for references see Kligman & DeLamater, 1950; Netto, 1955; Seeliger, 1958). In the study of sporotrichosis, complement fixation was inferior to other serological methods (Nordén, 1951). So far it has not been applied in the diagnosis of superficial fungus infections, but some promising results were obtained with deep-seated infections caused by dermatophytes. We have been able to detect complement-fixing antibodies as well as agglutinins and precipitins in a case of mycetoma of 20 years duration caused by *Monosporium apiospermum* (Seeliger, 1956).

Besides these methods, sensitized collodion particles, latex particles, sheep erythrocytes and resins have been used successfully in fungus serology. Further, fluorescein-stained antibodies have been used to trace antigenic material within different layers of fungus cells and within the tissues of the infected host.

Likewise the demonstration of fungistatic or fungicidal antibodies, as used by Janke (1955), belongs to this field. We found that certain batches of γ-globulin contained fungicidal antibodies against *C. albicans*, while normal sera did not.

Conclusions

Although there are certain *technical difficulties*, it seems to me that the interpretation of serological reactions with fungi is not always as complicated as with bacteria. Fungi apparently react as more or less species- or genus-specific. It also appears that many important species cannot be split into serotypes. One must keep in mind that microbial antigens are complex and usually consist of several fractions. A given fungus species, however, like *Candida albicans*, always shows a similar or identical antigenic make-up, but on the other hand *Escherichia coli* may be subdivided into more than 130 serological groups, according to differences in the somatic antigens.

There are some definite limitations in fungus serology: one of the greatest obstacles is the often encountered difficulty in the preparation of good antisera; another, to some extent, the production of stable antigens. It is hoped that growing interest in serological methods, as shown now by many mycologists, may result in still better methods of investigation.

What would then be my prognosis? I think that the application of serology offers a valuable additional tool for investigating phylogenetic relationships among fungi. Serological tests could, indeed, help in the solution of some difficult taxonomic problems. Last, but not least, further studies might be rewarding to the mycologist who tries to improve and accelerate his diagnostic work in the study of fungi as well as of pathologic conditions caused by, or associated with, these fascinating micro-organisms.

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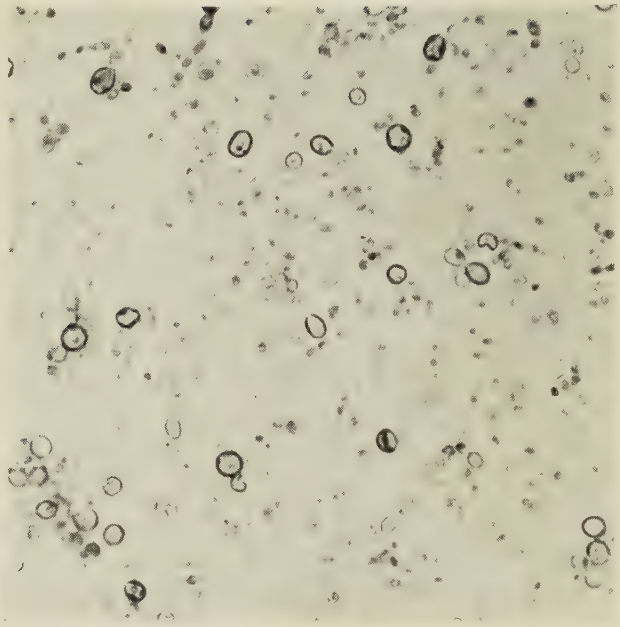

Fig. 1

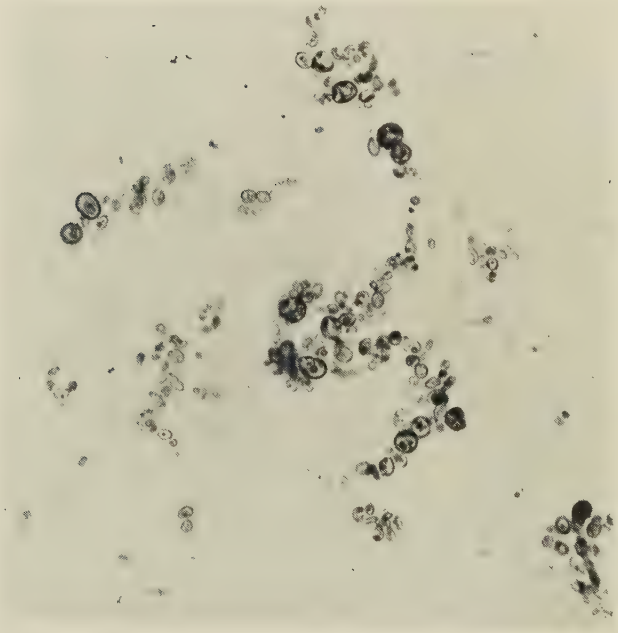

Fig. 2

(*Facing p.* 555)

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EXPLANATION OF PLATE 20

Fig. 1. Cover-slide preparation of yeast cells of *Pullularia bergeri* in the presence of 1:10 diluted antiserum of *P.* (*Cladosporium*) *werneckii*.

Fig. 2. Cover-slide preparation of yeast cells of *P. bergeri* in the presence of 1:10 diluted antiserum of *P. bergeri*.

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ANKISTROCLADIUM FUSCUM GEN.NOV., SP.NOV., AN AQUATIC HYPHOMYCETE

By ELIZABETH PERROTT

University College of North Staffordshire, Keele

(With Plate 21 and 1 Text-figure)

Ankistrocladium fuscum gen.nov., sp.nov., a submerged aquatic hyphomycete found growing on submerged twigs of *Quercus* sp., is described. The simple conidiophore is short, dark and one-celled, bearing a single apical conidium. Conidia are dark brown and multicellular. Each consists of a long, thick-walled, septate, curved, fusoid axis, hooked at the tip, bearing at the base 2 pairs of lateral branches of the same shape as the axis. One of the lower branches frequently branches again to produce a hexa-radiate spore; occasionally there may be seven or nine branches.

This fungus was found growing on submerged undecorticated twigs of *Quercus* sp. collected from water approximately 1 ft. deep at the margin of Ullswater, Westmorland, in May 1956. After collection the twigs were kept in a dish of distilled water at 3° C. At first only sterile, septate, fuscous hyphae grew from the broken ends of the twigs. The striking dark brown spores were not seen until March 1957 when fruiting hyphae of *Monoblepharis macrandra* had also developed on the substratum.

The fuscous, septate mycelium, composed of cells 14–16μ long × 4–6μ wide, occurs on the surface of the substratum. The conidiophores are approx. 36μ long, unbranched, fuscous, unicellular and lateral. The spores are borne singly and apically on the conidiophore from which they readily become detached.

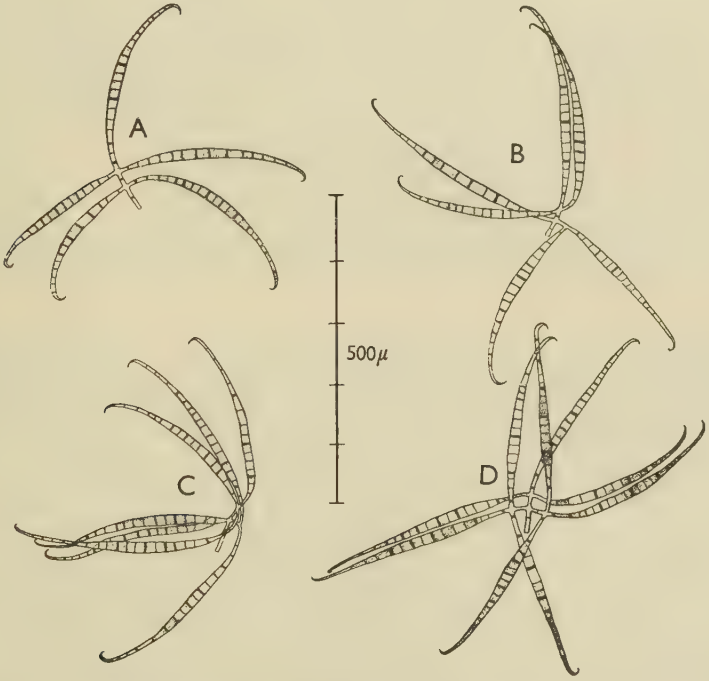
Each thick-walled, dark brown spore is a branch system consisting of a main axis with usually 2 pairs of opposite lateral branches, giving it a penta-radiate form (Text-fig. 1A and Pl. 21, fig. 1). The branches are distinctly septate, the majority of the septa being of the same thickness as the spore wall, but these are interspersed by septa which are twice as thick (Text-fig. 1 and Pl. 21). The main curved fusoid axis, composed of 16–24 cells, is 375–480μ long and approx. 12μ wide tapering to 2–4μ at the tip and base, the apical cell tapering and curving to form a hook. The branches, which are also fusoid, curved, and hooked at the tip, are 300–390μ × 8–12μ and have 10–24 cells. One of the lower branches frequently branches again near its point of attachment to the axis to produce a hexa-radiate form (Text-fig. 1B). Occasionally seven- (Text-fig. 1C) and nine-branched spores (Text-fig. 1D and Pl. 21, fig. 2) are produced by each of the lower branches forming one or two secondary laterals. In nature the spores are not in a single plane.

It has been suggested by Ingold (1942) that the biological value of the branched spore in an aquatic environment is likely to be the part it plays in

aiding the spore to come to rest on a suitable substratum. In this connexion it is interesting to note the distinct hooks which terminate each branch of the spore. The many-branched spores of previously described aquatic hyphomycetes (Ingold, 1942, 1959) are thin-walled and hyaline, in contrast to the thick-walled fuscous spore of *Ankistrocladium*.

Ankistrocladium gen.nov.

Fungus aquaticus submersus, mycelio septato, fusco. Conidiophorum simplex unicelluläre fert unum apicale conidium. Conidia fusca et multicellularia, consistentia ex longo, crassiore, septato, curvato, fusiformi axe qui unculo terminatur, quattuor ramulos, binos utrimque, laterales fert eiusdem formae ac axis. Ramuli interdum unus vel multiore ramuli genitis. Species typicus A. fuscum.



Text-fig. 1. *Ankistrocladium fuscum* sp.nov. A, a dark brown, multicellular conidium consisting of a septate, curved, fusoid axis, hooked at the tip, bearing 2 pairs of lateral branches; the penta-radiate spore is the most commonly occurring form; B, a hexa-radiate conidium, formed by one of the lower branches of a penta-radiate spore producing a secondary branch near its point of attachment to the axis. This spore form occurs frequently. C, a seven-branched conidium, formed by the lower pair of branches of a penta-radiate spore each producing a single secondary branch; D, a nine-branched conidium formed by the lower pair of branches of a penta-radiate spore each producing a pair of secondary branches.

Ankistrocladium fuscum sp.nov. (Text-fig. 1 and Pl. 21)

Fungus aquaticus submersus. Mycelium fuscum septatum consistens ex cellulis 14–16 μ longis, 4–6 μ latis, substrati in superficie invenitur. Conidiophora brevia (c. 36 μ longa), sine ramulis, fusca, unicellularia, et lateralia. Singula atque apicalia conidia sistunt in conidiophoris a quibus facile seiunguntur. Unum quodque conidium, crassius, fuscum, systema ramulorum, consistens ex principali axe ferente plerumque

quattuor ramulos laterales, binos utrimque, praebentes pentaradiatam formam. Conidium haud dubie septatum. Pleraque septorum eiusdem crassitudinis ac conidii paries, intermixta nonnullis septis duplicis crassitudinis. Principalis axis curvatus fusiformis consistit ex 16–24 cellulis, ex quibus apicalis cellula extenditur in unculum, qui est 375–480 μ longus et c. 12 μ latus et fit angustior usque ad 2–4 μ in termino atque in basi. Ramuli item fusiformes, curvati, cum unculo terminali 300–390 μ longi, 8–12 μ lati, inveniuntur, consistentes ex 16–24 cellulis. Unus ex inferioribus ramulis iterum ita diffunditur, prope locum ubi axi coniungitur, ut hexaradiatam formam praebeat. Interdum septem-ramula et novem-ramula conidia praebentur ab uno quoque inferiorum ramulorum ita ut unus aut duo secundarii ramuli praebeantur. Ramuli non in uno plano dispositi sunt.

Habitat: In submersis indecorticatis ramulis Quercis, iacentibus in aqua ad unum pedem alta in margine Ullswater lacus, Westmorland, Angliae, May 1956. Typus: Herb. I.M.I. 78682.

My thanks are due to Prof. J. M. T. Charlton of the University College of North Staffordshire for his invaluable assistance with the Latin diagnosis and the choice of a name for this genus, to Prof. C. T. Ingold of Birkbeck College for his interest and advice, and to Mr G. Burgess who took the photographs.

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EXPLANATION OF PLATE 21

Fig. 1. One conidium of *Ankistrocladium fuscum* showing the main axis with four lateral branches arranged in pairs to produce a penta-radiate form, the most common form found. × 300.

Fig. 2. A nine-branched conidium of *A. fuscum*, produced by each of the lower branches forming a pair of secondary laterals. × 300.

(*Accepted for publication* 11 *December* 1959)

Fig. 1

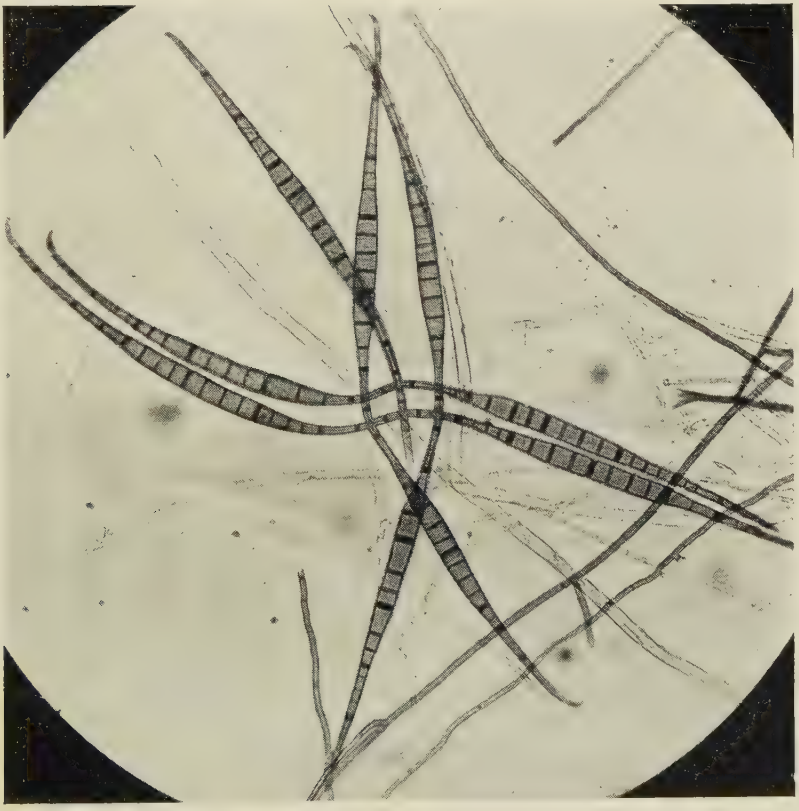

Fig. 2

(*Facing p.* 558)

Trans. Brit. mycol. Soc. **43** (3), 559–570 (1960).

STUDIES IN VESICULAR-ARBUSCULAR ENDOPHYTES

II. ENDOPHYTES IN THE PTERIDOPHYTA, WITH SPECIAL REFERENCE TO LEPTOSPORANGIATE FERNS

By PAMELA M. HEPDEN*

Department of Botany, University of Bristol

(With 2 Text-figures)

Previous records of the presence of vesicular-arbuscular endophytes in the gametophytes and in the roots of sporophytes of the Pteridophyta are listed and show their widespread occurrence. The present study confirms many of these records and establishes several new ones. Natural infection of prothalli of a number of Leptosporangiate ferns is reported, indicating that the condition is more frequent than the single previous record would suggest. The endophytes of ferns are similar in appearance to those in *Allium ursinum* and other Phanerogams. Isolates from the roots of sporophytes and from prothalli were all identified as a species of *Pythium* resembling but not identical with *P. ultimum*. They showed a general similarity among themselves and a close resemblance to isolates from similarly infected roots of *Allium* spp.

Prothalli of *Phyllitis scolopendrium* were grown on sterilized soil, on sterilized sand or on agar media, with and without the addition of the *Pythium* isolate. In inoculated soil the prothalli were invaded and a typical vesicular-arbuscular endophyte developed. Under other conditions inoculated prothalli were invaded and destroyed, or invasion was limited to the rhizoids. In experiments with sand culture moistened with nutrient solution, supplies of carbohydrate and of nitrate were varied. When 2 % sucrose was supplied at the beginning of the experiment, development of the prothalli was retarded whilst growth of the fungus was much improved, and the prothalli were killed. Experiments in which nitrate concentration was varied were inconclusive. Prothalli grown on uninoculated media remained free from infection.

Roots of young sporophytes of *P. scolopendrium* developing from prothalli grown in sterilized soil inoculated with *Pythium* sp. (isolated from *P. scolopendrium* or *Dryopteris filix-mas*) readily became infected, and a typical vesicular-arbuscular endophyte subsequently developed, provided that the time of inoculation and nutritional factors were suitable. Infection of the roots took place directly from the soil or other culture medium, and not via the infected parent prothallus. Control plants were uninfected.

It is concluded that the *Pythium* isolates are identical with the endophyte in *P. scolopendrium*, but that their establishment in the host in the vesicular-arbuscular form depends on a rather limited range of environmental conditions.

Vesicular-arbuscular endophytes have been described for a wide range of plants. Hawker, Harrison, Nicholls & Ham (1957) describe the isolation of *Pythium* from infected roots of *Allium ursinum* and some other plants, including the sporophyte of a species of *Dryopteris*. A typical vesicular-arbuscular endophyte developed in roots of *Allium cepa* grown in naturally infected soil and in sterilized soil inoculated with these isolates.

* Present address: Mrs K. Joy, Department of Botany, Imperial College of Science and Technology, London, S.W. 7.

A number of observers have reported the presence of similar endophytes in the prothalli of various Pteridophyta. Boullard (1957) describes an extensive survey of the incidence of mycorrhizas in the Pteridophyta. Records of vesicular-arbuscular endophytes in the literature are summarized below.

Previous records of vesicular-arbuscular endophytes in the Pteridophyta (excluding negative records)

Lycopodiales

(*a*) *Sporophytes.* Sixteen species of *Lycopodium* (references in Kelley, 1950). *Selaginella spinulosa* (Bruchmann, 1897); other species of *Selaginella* lacking root hairs (Janse, 1897); sporophytes of Lycopodiaceae with variable infection (Boullard, 1957).

(*b*) *Gametophytes.* Generally present in chlorophyll-less prothalli of *Lycopodium* spp. (Bruchmann, 1885; Treub, 1885; Burgeff, 1938; Boullard, 1957); massive, partly green prothalli also infected (Boullard, 1957).

Psilotales

(*a*) *Sporophytes. Psilotum triquetrum* rhizome (Solms-Laubach, 1884; Janse, 1897; Bernatsky, 1899); *Tmesipteris* sp. (Dangeard, 1891); infection in all species examined (Boullard, 1957); *Paleomyces*, a fungus in the roots of the Rhynie fossils (Kidston & Lang, 1921), resembles the endophytes of modern ferns.

(*b*) *Gametophytes. Psilotum* sp. (Holloway, 1920; Lawson, 1917).

Filicales (*Eusporangiatae*)

Ophioglossaceae

(*a*) *Sporophytes. Ophioglossum vulgatum* (Russow, 1872; Bruchmann, 1904); *O. pendulum, O. moluccanum* (Campbell, 1907); *O. simplex* (Bower, 1904); twelve species of *Botrychium* (Grevillius, 1895); *B. lunaria* (Kühn, 1889); *Helminthostachys* sp. (Farmer & Freeman, 1899); infection in all species examined (Boullard, 1957).

(*b*) *Gametophytes.* All species of *Botrychium* and *Ophioglossum* examined contain endophytes (Hofmeister, 1862; Mettenius, 1856; Campbell, 1895, 1907; Bruchmann, 1904, 1906; Jeffrey, 1898; Lyon, 1905; Boullard, 1957).

Marattiaceae

(*a*) *Sporophytes.* All infected (Boullard, 1957).

(*b*) *Gametophytes.* All infected (Boullard, 1957).

Filicales (*Leptosporangiatae*)

Osmundaceae

(*a*) *Sporophytes. Osmunda regalis* (Van Tieghem, 1870); *Todea barbara* (Cribbs, 1920); all infected but infection slight (Boullard, 1957).

(*b*) *Gametophytes.* Two species of *Osmunda* (Campbell, 1908); infection variable (Boullard, 1957).

Schizaceae

(*a*) *Sporophytes*. All infected (Boullard, 1957).
(*b*) *Gametophytes*. Infection slight and variable (Boullard, 1957).

Gleicheniaceae

(*a*) *Sporophytes*. All infected (Boullard, 1957).
(*b*) *Gametophytes*. Five species with endophytes (Campbell, 1908); all infected (Boullard, 1957).

Cyatheaceae

(*a*) *Sporophytes*. *Cyathea* sp. (Janse, 1897); three species with endophyte (Asai, 1934); all infected (Boullard, 1957).

Hymenophyllaceae

(*a*) *Sporophytes*. *Hymenophyllum* always infected, *Trichomanes* with infection variable (Boullard, 1957).
(*b*) *Gametophytes*. *Hymenophyllum*, infection 'superficial' (Boullard, 1957).

Polypodiaceae

(*a*) *Sporophytes*. *Cystopteris fragilis*, *Onodea sensibilis*, *Pteridium aquilinum* (Lohman, 1927); *Adiantum pedatum* (Lohman, 1927; Doak, 1927); *A. flabellatum*, *Nephrolepis* sp., *Blechnum* sp. (Asai, 1934); *Polypodium feei* (Faber, 1925); infection sporadic (Boullard, 1957); *Dryopteris* sp., endophyte isolated and identified as *Pythium ultimum* (Harrison, 1957).
(*b*) *Gametophytes*. *Cheiropleuria bicuspis* (Nakai, 1933).

Distribution and morphology of endophytes in certain Pteridophyta

Collection and preservation of ferns

Sporophyte and gametophyte plants of native ferns were collected from various sites in Cumberland, Bristol and Somerset. A few specimens were obtained from the fernhouse in the Botanic Garden at Bristol University. Material was usually examined when fresh, but when this was not possible roots and prothalli were washed thoroughly and fixed in a modification of Navashin's fluid (Sass, 1940).

Methods of examination

Sectioning of the tough fine roots of ferns was impracticable. A maceration technique based on that described by Mangin (1895) gave suitable preparations. Pieces of root or prothalli were boiled in 2 % aqueous potassium hydroxide, for 3 min. for prothalli and 10–15 min. for older roots, dissected under a binocular microscope to expose deep-seated tissues, and stained in 2 % aniline blue in lactic acid for at least 12 hr. Comparison with sections of unmacerated material showed that the treatment did not appreciably change the appearance of hyphae, vesicles or arbuscules.

Endophytes in the roots of sporophytes

The incidence of infection in sporophytes of species examined by the writer is given in Table 1.

The fungus sometimes entered the root through the root hairs (Fig. 1, ii), but more often it entered directly through the piliferous layer, frequently forming an appressorial swelling at the point of entry (Fig. 1, iv). The infected regions were well defined, and were usually zones of cortex one or two cells deep, directly outside the stele. Meristematic and stelar tissues were never invaded.

Table 1. *Summary of the incidence of endophytic infection in Pteridophytes examined by the writer*

Class	Family	Species	Incidence of infection and site of collection
Lycopodinae	Selaginellaceae	*Selaginella kraussiana* (S)	++(F)
Isoetinae	Isoetaceae	*Isoetes* sp. (S)	o (W)
Filicinae (Eusporangiatae)	Ophioglossaceae	*Ophioglossum vulgatum* (S)	++(BB)
		Botrychium lunaria (S)	++(BB)
		B. lunaria (G)	++(BB)
(Lepto-sporangiatae)	Polypodiaceae	*Adiantum capillus-veneris* (S)	++(F)
		A. capillus-veneris (G)	++(F)
		Asplenium bulbiferum (S)	++(F)
		A. bulbiferum (G)	++(F)
		A. ruta-muraria (S)	o (L), o (C)
		A. trichomanes (S)	++(L), o(GC), o (H), ++(BC)
		Athyrium filix-femina (S)	++(LW)
		Blechnum spicant (S)	++(LF)
		Ceterach officinarum (S)	o (CM)
		Cryptogramma crispa (S)	++(LF)
		Dryopteris filix-mas (S)	++(H), ++(GC)
		D. filix-mas (G)	++(H), ++(BC)
		Phyllitis scolopendrium (S)	++(GC), ++(BB), ++(LW), o (CM), ++(BC)
		P. scolopendrium (G)	+(B), +(GC)
		Polypodium vulgare (S)	o (LW), o (GC), +(D), ++(BC)
		Pteridium aquilinum (S)	++(BB), ++(BC)
		P. aquilinum (G)	+(BB)
		Pteris critica (S)	o (F)
		P. critica (G)	o (F)
		Thelypteris palustris (S)	++(Gl)
	Hymenophyllaceae	*Hymenophyllum* sp. (S)	o

Key. S = Sporophyte, G = gametophyte. ++ = most roots or rhizoids infected, + = a few roots or rhizoids infected, o = no infection. B = Blaise Castle, Bristol; BB = Braunton Burrows, N. Devon; BC = Burrington Combe, Somerset; C = Clifton, Bristol; CM = Chew Magna, Somerset; D = Dundry, Nr. Bristol; F = fernhouse, Bristol University; GC = Cleeve, Somerset; Gl = Glastonbury, Somerset; H = Hiatt Baker Garden, Bristol; L = Langford, Somerset; LF = Loughrigg Fell, Cumberland; LW = Leigh Woods, Bristol; W = Windermere, Cumberland.

Characteristic aseptate or sparsely septate hyphae, irregular in diameter and often containing oil drops, were always present, growing longitudinally through the roots. Vesicles, when present, varied considerably in size and

shape (Fig. 1, i and iii) and were intercellular, intracellular or extramatrical. The large vesicles figured for *Pteridium aquilinum* resemble closely those in the roots of grasses, investigated by Nicolson (1959). Pelotons and arbuscules were frequent. In older roots the later stages of digestion of arbuscules were common. In tissues normally rich in starch (e.g. root cortex of sporophyte of *Botrychium lunaria*), infected cells lacked starch but were rich in oil and proteinaceous material.

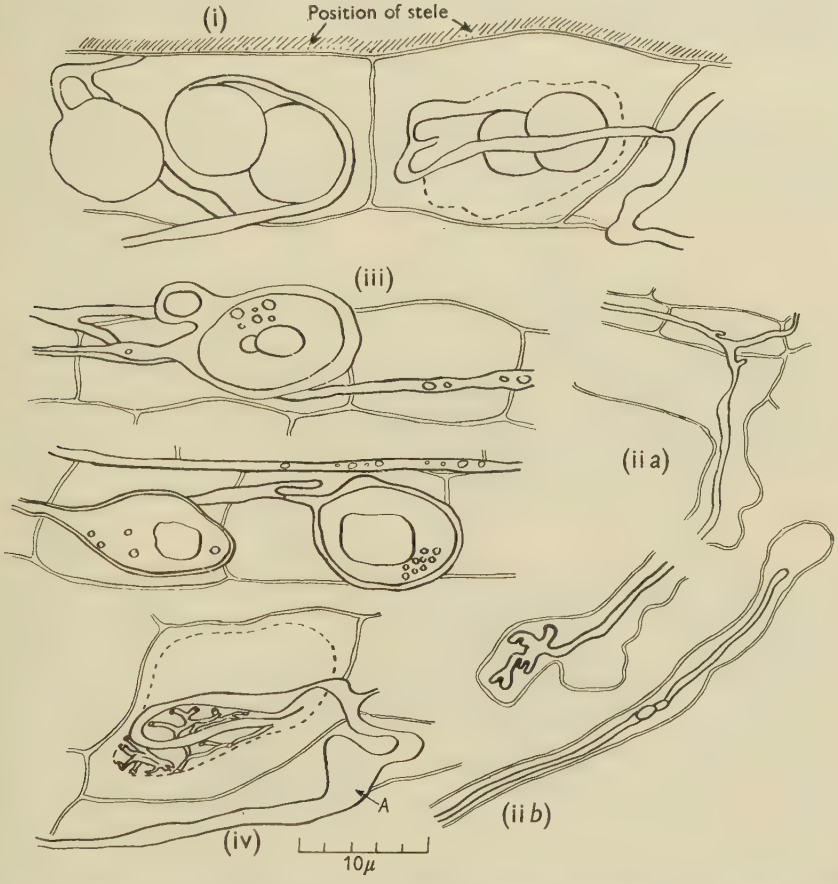

Fig. 1. Vesicular-arbuscular endophytes in ferns. Natural infections in sporophyte roots. (i) *Adiantum capillus-veneris*, intracellular vesicles in root cortex cells. (ii) Infected root hairs: (*a*) *Cryptogramma crispa*; (*b*) *Dryopteris filix-mas*. (iii) Intercellular vesicles in root cortex of *Pteridium aquilinum*. (iv) Appressorial swelling (*A*) on root surface and arbuscule in root cortex cell in *A. capillus veneris*; broken line indicates the outline of the arbuscule.

Not all specimens of any particular species were infected. Infection also varied with the age of the plant, the earliest formed roots often being heavily infected and later ones more sporadically so. Lang (1902) reported that in *Ophioglossum pendulum* the first three roots of the sporophyte were infected, and all subsequent roots remained uninfected, but no such definite

distinctions have been observed in the present study. There was no correlation between incidence of infection and environmental factors, such as soil type and moisture content.

Endophytes in gametophytes

Gametophytes examined by the writer and containing endophytes are also listed in Table 1. Prothalli of the Eusporangiatae were regularly infected but those of the Leptosporangiatae more rarely so. In the Leptosporangiatae, invasion was always through a rhizoid, beyond which the

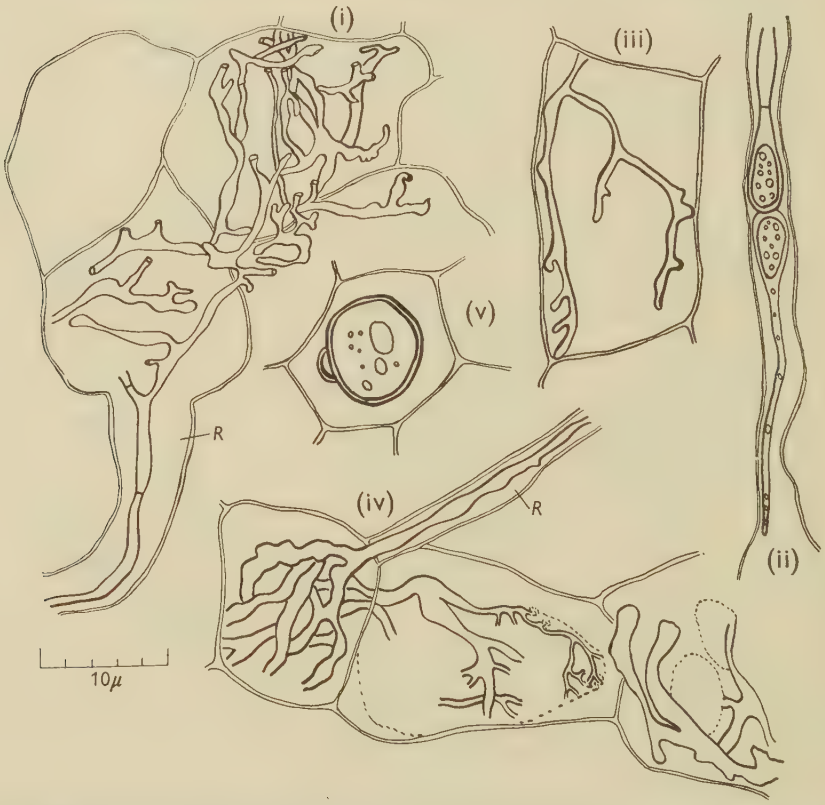

Fig. 2. Vesicular-arbuscular endophytes in ferns. Infected gametophytes. (i) *Dryopteris filix-mas*, naturally infected rhizoid (*R*) and young arbuscule in thallus cell. (ii) *Phyllitis scolopendrium*, infected rhizoid (*R*), 1 month after inoculation with an isolate of *Pythium* in pure culture. (iii) *P. scolopendrium*, branching hyphae in thallus cell, 2 months after inoculation with *Pythium* in pure culture. (iv) *P. scolopendrium*, infected rhizoid (*R*) and arbuscules in thallus, 3 months after inoculation with *Pythium* in pure culture. (v) *P. scolopendrium*, vesicle in thallus cell, 3 months after inoculation with *Pythium* in pure culture.

fungus seldom penetrated. Vesicles were occasionally seen inside rhizoids and more rarely in thallus cells. When thallus cells were invaded typical arbuscules developed (Fig. 2, i). The cells of the basal cushion of the prothallus were most frequently infected; peripheral thallus cells and those

adjacent to reproductive organs never contained arbuscules. Intercellular hyphae, vesicles and arbuscules were morphologically similar to those in sporophyte roots.

Isolation and identification of the endophyte

Fungi were isolated from roots and thalli by Harrison's section-embedding technique (Harrison, 1955). A species of *Pythium* was isolated repeatedly from sporophytes of *Dryopteris filix-mas* and *Phyllitis scolopendrium* growing in a number of localities, and from the gametophyte of *Dryopteris filix-mas*, thus confirming Harrison's (1957) result.

Isolates of the *Pythium* sp. from *P. scolopendrium* produced more abundant white aerial mycelium on potato-extract agar than did those from either generation of *Dryopteris filix-mas*. The latter produced flatter colonies with rather more vesicles. Microscopic and colonial characters of all isolates in pure culture closely resembled those of isolates from some monocotyledons (Hawker *et al.* 1957).

Inoculation of prothalli and young sporophytes of *Phyllitis scolopendrium* with *Pythium* isolates

The establishment of the vesicular-arbuscular condition by the inoculation of fern prothalli and young sporophytes with the *Pythium* isolates was attempted by a number of methods. Growth of prothalli under aseptic conditions was difficult, but eventually a satisfactory method was evolved which gave positive results under suitable conditions.

Methods

Surface sterilization of fern spores. A procedure based on that of Schwabe (1951) was adopted. Dry sporophylls were pressed between pieces of clean dry paper for several days, until a number of spores had fallen from their sporangia. These spores were removed with a dry brush, washed in twelve changes of sterile water, and centrifuged each time to remove light (probably non-viable) spores and fragments of sporangia.

Media and other environmental conditions. Spores were allowed to germinate in Petri dishes containing a shallow layer of sterilized Knop's solution (KNO_3, 1 g.; $MgSO_4$, 1 g.; $Ca(NO_3)_2$, 3 g.; K_2HPO_4, 1 g.; distilled water 1 l.). Four weeks after sowing, when most spores had produced a few green cells, the sporelings were transferred from the dishes of Knop's solution to the various solid media. In some experiments, surface-sterilized spores were sown directly on to the solid experimental medium.

The most satisfactory medium for growth of both prothalli and *Pythium* was sterilized soil (steamed for 1½ hr.). Natural soil (from Cleeve, Somerset) and John Innes's potting soil (7 parts loam, 3 parts peat, 2 parts sand) were used. Moore's agar medium (NH_4NO_3, 0·5 g.; KH_2PO_4, 0·2 g.; $MgSO_4$, 0·2 g.; $CaCl_2$, 0·1 g.; agar, 10 g.; distilled water, 1 l.) or sand moistened with Knop's solution were used in some experiments.

Liquid and agar cultures were kept at 22° C., with alternating 12 hr. periods of darkness and light. Preliminary experiments with sand and soil

were also kept under these conditions, but the main experiments were carried out in a temperate glasshouse. Flower pots were stood in individual shallow glass dishes, and watered regularly with sterile tap water. Nutritional conditions, including carbohydrate and nitrate supply, and the time of inoculation with the fungus, were varied.

Fungal inocula. Conical flasks (500 ml.) containing 50 g. of a mixture of 3–5 parts oatmeal to 95 parts acid-washed sand + 5 ml. distilled water were plugged with cotton wool, autoclaved and inoculated with Harrison's (1957) isolate from the sporophyte of *Dryopteris* sp., with the writer's isolate from the gametophyte of *D. filix-mas* or with one of a number of isolates from sporophytes of *Phyllitis scolopendrium* obtained from different sites in the West of England. Cultures were incubated at 20° C. for 1–2 weeks.

Two to three g. of the sand and oatmeal culture were added to each vessel of soil or sand, 4 weeks prior to planting out of spores or prothalli, or at the time of sowing, or later (i.e. after the prothalli had become established).

Samples of inoculated and of control soils were tested for the presence of *Pythium* by baiting with hemp seed during the experiments.

Inoculation of prothalli and sporophytes grown in soil

Small flower pots containing sterilized natural soil or potting soil were sown with spores of *P. scolopendrium*. The time of inoculation of the soils with *Pythium* was varied. The earliest inoculations were made 1 month before the fern spores were sown. Other batches were inoculated at monthly intervals after this. No additional nutrients were supplied. There were approximately ten prothalli per pot, and three pots per treatment. Prothalli were sampled at monthly intervals and details of size, morphology, development of sex organs and microscopic details of fungal infection were recorded.

Effect of the fungus on the germination of fern spores. In flasks and pots inoculated with *Pythium* prior to germination of the fern spores, percentage germination was considerably reduced. This thinning out resulted in fewer and larger prothalli than in uninoculated controls.

Effect of the fungus on young prothalli. Prothalli which were already established prior to inoculation of the soil were seldom invaded, even when large amounts of mycelium were ramifying amongst the rhizoids. Penetration of the latter was frequent.

When germinated fern spores were planted in sterilized soil which had been inoculated with the fungus 1 month earlier, a condition resembling the natural endophytic one developed. One month after transfer of prothalli into such infected soil, there was some penetration of rhizoids (Fig. 2, ii). After a further month, branched aseptate hyphae, typical of the early stages of arbuscule development (Fig. 2, iii), were present in a number of thallus cells of apparently healthy individuals. At this stage *Pythium* was readily re-isolated from infected thalli.

After 3 months, most of the infected prothalli had given rise to sporophytes, and were themselves beginning to disintegrate. Mature arbuscules were present in many disintegrating prothallus cells (Fig. 2, iv). Others contained vesicles (Fig. 2, v). Successful re-isolation was not achieved at

this stage, but work in progress in this laboratory shows that isolation from mycorrhizal higher plants is similarly difficult when the fungus has reached the mature arbuscular state. Comparison with uninoculated controls, which all remained free of infection, showed that death of old prothalli was not accelerated and development of sex organs and sporophytes was unaffected by the presence of the fungus in the endophytic condition.

Effect of the fungus on young sporophytes. Sporophytes developed from prothalli, in the experiment described above, in both uninoculated and inoculated soils. As in the experiment with prothalli, batches of previously uninoculated soils were inoculated at monthly intervals. When sporophytes in previously inoculated soils were approximately 1 month old, their roots were surrounded by wefts of hyphae. The extreme tips of the roots were not invaded but hyphae were running longitudinally through the cortex behind the tip. Arbuscules developed later. Frequent but sporadic invasion of new roots as they developed was observed. Invasion of the sporophyte always took place directly from the soil and not through the tissues from the infected parent prothallus. Plants in uninoculated soil remained free of infection.

Pythium sp. was re-isolated from an infected sporophyte. It was repeatedly obtained from the inoculated soil by the hemp-seed baiting method. Uninoculated sterilized soil yielded no *Pythium* at the beginning of the experiments, and subsequent contamination by *Pythium* was rare.

Results of a parallel series of experiments with unsterilized potting soil were comparable. The mycorrhizal condition developed only in cultures inoculated with *Pythium*. Control plants remained free of invasion.

Experiments with agar cultures

Soil was too complex a medium for use in experiments on the effect of nutrition. Fern spores were sown on Moore's agar which was later inoculated with *Pythium* isolates. Prothalli were not visibly damaged by the fungus, although the rhizoids were penetrated. Arbuscules formed in the roots of sporophytes which developed from the prothalli. Uninoculated controls remained uninfected. Desiccation of the agar made it unsuitable for prolonged experiments.

Experiments with sand cultures

Sterilized sand moistened with Knop's culture solution or with modifications of this was placed in plugged, wide-necked flasks. Young prothalli were planted aseptically on the sand, which was kept moist by additions of sterile water. The nature and concentration of carbohydrates, nitrogen compounds and vitamins in the culture solution were varied. The times at which carbohydrate was supplied or inoculation with *Pythium* took place were also varied.

The condition most nearly approaching that of the naturally infected mycorrhizal prothalli was obtained when the fungus and 2·0 % sucrose were both added 1 month after transfer of the prothalli to the sand cultures from the liquid medium in which the fern spores had germinated. Four weeks after inoculation, the fungal hyphae had penetrated the rhizoids

and many extramatrical vesicles were present. Thallus cells adjoining the infected rhizoids were killed. Sucrose retarded growth of prothalli in control cultures, but the fungus was unable to grow through the sand without the addition of sugar. It might be possible, by further adjustment of the amount and time of addition of sucrose, to induce the development of the typical vesicular-arbuscular condition in prothalli in sand cultures, as in soil. Where sporophytes developed, these readily became mycorrhizal under the conditions of the experiment. Uninoculated controls all remained free of infection.

Experiments in which the concentration of nitrate was varied, or various vitamins were added, gave inconclusive results.

Discussion

The identity of the vesicular-arbuscular endophytes occurring in a wide range of green plants has received much attention recently. The present study shows conclusively that, under certain conditions, a species of *Pythium* is able to invade and develop into a typical endophyte in the prothalli and sporophyte roots of certain ferns. The morphology and behaviour of the *Pythium* isolated from ferns resemble those of isolates from mycorrhizal roots of *Allium* spp. and some other plants.

Evidence is accumulating, however, that species of *Endogone* (Mosse, 1956; Nicolson, 1959; Dowding, 1959) produce a vesicular-arbuscular condition in the roots of many plants. Attempts to isolate *Endogone* from roots and to grow it in pure culture have not so far been successful. In the present study, isolation of an endophytic fungus from fern roots was not invariably achieved, and it may well be that some of these failures were due to the presence of endophytes other than *Pythium* and that these cannot be isolated by the section-embedding technique.

Fern prothalli were more difficult to grow in culture than were sporophytes. The delicate prothalli were readily killed by the fungus but nevertheless a mycorrhizal condition was achieved by inoculation with the *Pythium* isolate under certain critical conditions. Such a balanced relationship was more readily obtained with inoculated sporophytes. Natural infection also is more frequent in the sporophyte than in the prothallus among leptosporangiate ferns. The normal life span of the prothallus may be too short to allow the development of mature arbuscules. Moreover, the experiments indicate that the conditions permitting the development of a typical mycorrhizal relationship between *Pythium* and a fern prothallus are limited, and that outside these limits the fungus either fails to penetrate beyond the rhizoids or is able to kill the prothallus. These experiments, and the observation that not all individuals of a particular host species are infected, even when growing close to one another, offer an explanation of the contradictory nature of some earlier records, and support the interpretation of the vesicular-arbuscular mycorrhizal condition as one of controlled parasitism.

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PORIA CARBONACEA (BERK. & CURT.) COOKE AS A CAUSE OF TIMBER ROT IN JAMAICA

By JOY ROYES*

Ministry of Trade and Industry, Jamaica, W.I.

AND A. R. LOVELESS†

University College of the West Indies, Jamaica, W.I.

Poria carbonacea (Berk. & Curt.) Cooke, a tropical brown-rot fungus, is recorded as causing decay of structural timber (*Brosimum alicastrum* Sw.) in recently built houses in Jamaica. The outbreaks showed several features in common with dry rot caused by *Merulius lacrymans*, but the mycelial sheets on the surface of infected timber were not seen to form mycelial strands. The sporophore and cultural characters of *P. carbonacea* are described. The optimum temperature for growth is about 31·5° C. and the minimum relative humidity for development of mycelium on agar is about 90% at 30° C. The fungus is comparable with other well known wood-destroyers in its ability to decay wood of *B. alicastrum*.

In tropical countries fungal decay of the woodwork in houses is of secondary importance to termite damage, and consequently authentic records of its occurrence are rare. The observation of two striking cases (and a verbal report of a third) of 'dry rot' caused by *Poria carbonacea* (Berk. & Curt.) Cooke (not to be confused with *P. carbonica* Overholts) in Jamaica between 1953 and 1955 is therefore considered worthy of record.

Both outbreaks occurred in recently built houses (less than 2 years old) and, by the time they had come to our notice, the owners were demanding that the builders should repair the damage. In each instance the infected structural timber was behind plaster and, although portions of the sporophores were removed from both outbreaks, the owner of only one of the houses would allow us to remove a sample of the infected timber. Unfortunately, even this outbreak could not be investigated in detail because the builder, when he saw that a sample of the infected timber had been removed, refused to repair the damage if further outside interference took place. The owner, although previously very co-operative, therefore felt that, in his own interests, he could not allow any further investigation of the outbreak by us.

The details of this outbreak, as reported by the owner, are as follows. The fungus was first seen about 5 October 1953 (at the beginning of the rainy season), only 6 months after the house had been completed and occupied, when a small resupinate sporophore appeared in a bedroom on an outer

* Present address: c/o Institute of Animal Genetics, King's Buildings, West Mains Road, Edinburgh 9.

† Now at University College of Rhodesia and Nyasaland, Salisbury, Southern Rhodesia.

wall. In the words of the owner 'This was removed after the lapse of a day and the spot sprayed with Pyrethex and wiped with Dettol, and it did not return. A second outgrowth appeared nearby on or about 11 October, and was allowed to remain for about a day and a half before it too was taken off and the affected area wiped with soap and water. At the same time a small outgrowth from the outlet of an electric plug was also discovered, but after being removed it did not return. On or about 18 October a growth of the fungus started on top of the shower wall in the bathroom, and was allowed to remain for about 2 days before being taken off. In the space of another one and a half days it returned and was again removed. By 30 October, when you first visited the premises, the fungus had appeared and had been removed at least three times.'

As a result of three visits made between 30 October and 16 November (when, for the reason already given, the 'field' side of the investigation came to an untimely end) the authors were able to make the following general observations on the outbreak. There was no difficulty in accounting for its origin. The house, a bungalow, was constructed around a framework of stout wooden posts that were embedded in the concrete of the foundations. The posts were of *Brosimum alicastrum* Sw. (breadnut), one of the 'local hardwoods' (a trade-name used in Jamaica for several close-grained, locally grown timbers), and were not treated with a wood preservative on account of their supposed durability. By contrast, the imported softwood used in the construction of the cupboards, etc., was treated as a protection against termites. In view of the fact that there had been no leakage of water on the premises since their completion, coupled with the short time interval before the fungus became active (i.e. only 6 months after the bungalow was completed), it seems certain that the outbreak must have stemmed from the use of infected timber in the initial construction of the house, rather than from the germination of spores on the surface of the timber as a result of the penetration of dampness. This conclusion is supported by the fact that the 6 months preceding the outbreak cover a relatively dry period of the year in Jamaica.

The outbreak showed many features in common with outbreaks caused by *Merulius lacrymans* (Wulf.) Fr. Not only are the sporophores of *P. carbonacea*, apart from being darker brown in colour, similar in general appearance to those of *M. lacrymans*, even to the extent of having a conspicuous white or greyish margin, but the phenomenon of 'weeping' (though not evident at the time of our visits) also apparently occurs as shown by the presence of moisture trails left on the wall below one of the larger fructifications. Furthermore, the mycelium has the ability to penetrate plaster, concrete and brickwork for distances of at least 5 cm. Although functional sporophores developed only on the inside surface of affected walls, small erumpent masses of mycelium, not more than 1 mm. high, were located at corresponding positions in crevices on the outside of the wall; these mycelial cushions never developed further, presumably because they dried up as a result of exposure to the sun. Removal of the plaster behind one fructification in order to expose the infected post, revealed the presence of a well-developed mycelial sheet of a similar brown colour to the sporophore, on the surface of the timber. Although about 1 m. of the post was exposed

(and the mycelial sheet still continued) there was no indication of the formation of mycelial strands, which are such a characteristic feature of *M. lacrymans*. It would seem therefore that *P. carbonacea* has more limited powers of spread than has *M. lacrymans* and should consequently be easier to control. No cracks were visible in the wood of this infected post, but small samples sawn off along its length showed that the wood was discoloured to depths varying from 2 to 10 mm. below the mycelial sheet.

The rapidity with which *P. carbonacea* can form sporophores, once the mycelium is well established in wood, has already been mentioned in the verbal report of the owner, quoted above. A particularly striking example of this rapidity of growth under favourable conditions is provided by the following further observation on the present outbreak. At the time the fungus was active on top of the shower wall in the bathroom, a further outbreak occurred in a linen cupboard in the same room. This cupboard was opened at 3- to 4-day intervals for the removal of clean towels. During the time that elapsed between the shutting of the cupboard, when there were no obvious signs of the fungus, and the next time it was opened, the fungus had developed a conspicuous sporophore, measuring about 10 cm. in diameter, on the cupboard wall and mycelium was spreading actively through the towels, which were ruined.

Although only two cases of *P. carbonacea* causing rot of the structural timber in houses have been seen by the authors, the potential importance of this fungus in tropical countries is immense. It is conceivable that 'the leprosy of the house' referred to in the Bible (Lev. ch. xiv, *v.* 34 *et seq.*) was caused by this fungus, because the average temperature of Palestine is too high for the growth of *M. lacrymans*. Partly because of its potential importance, and partly because so little seems to be known about the fungus, the authors decided to make a laboratory investigation of *P. carbonacea* as a cause of timber rot, to supplement the 'field' data described above. The fungus was investigated along the lines adopted by Cartwright & Findlay (1946), in their treatment of the major wood-rotting fungi of Great Britain.

Occurrence of *Poria carbonacea*

Apart from the short type-description of *P. carbonacea*, based upon a collection made by C. Wright from a log in Cuba, there seems to be no published literature on this fungus. There are, however, two specimens additional to the type in the Kew Herbarium, one from a dead branch of *Albizia gummifera* (Gmelin) C. A. Smith in Sierra Leone [Coll. F. C. Deighton (M. 3520), at Njala (Kori), 30:1:50], and another from Brazil [Coll. J. R. Weir (det. in error as *P. carbonaria* Bres.), Fungi of the Amazon River Drainage no. 66857, at Rio Ouro Preto, Matto Grosso, 20:9:23]. Although in Jamaica *P. carbonacea* has so far only been reported as a fungus in buildings, it is clear from these other records that it also occurs in the field. The limited data of these records suggest that the fungus is normally a saprophyte on logs and on the dead branches of living trees, but the evidence is not sufficient to indicate whether or not it can become parasitic. It is noteworthy that it has been found in both the Old and New World Tropics.

The sporophore

A typical fruit-body of *P. carbonacea* assumes the form of a flat plate, 5–6 mm. thick at maturity, and varying in diameter from a few cm. to about 1 m. (The last figure is based on a fructification, approaching this size, that had developed at the back of a cupboard on the premises of the outbreak and about which no other information could be obtained.) The mycelial pad that is a precursor of a sporophore is either white or greyish in colour, but as it spreads laterally and develops into a functional sporophore, it rapidly turns brown, except at the growing margin which remains white or greyish. The configuration of the hymenial layer largely depends on the contour of the surface on which the fruit-body develops. On a flat surface, whether horizontal or vertical, the hymenium consists of shallow pores, mostly more or less circular in outline and about 0·7 mm. in diameter. When growing from a vertical position to the underside of a horizontal surface, or even when growing luxuriantly on a vertical surface, the hymenial folds may become elongated into teeth-like projections (cf. the 'stalactite' form of *M. lacrymans*). When fresh the sporophore is tough and corky (but not woody) in texture, though on drying it becomes hard and brittle; hence the specific epithet '*carbonacea*', although descriptive of a dried herbarium specimen, is not appropriate to the living fungus. Since fruit-bodies will develop in closed cupboards and similar situations, it seems that little or no light is required for their formation.

The spores are colourless, elliptical, and measure 6·5–7·0 × 2·5 μ.

Characters of the rot

P. carbonacea causes a brown rot. Even in an early stage of attack, the fungus causes a pronounced brownish coloration in wood on which it is growing. At a later stage shrinkage cracks develop along the grain, but there are no cross-cracks as in wood decayed by *M. lacrymans*. The mycelial sheets on the surface of decayed wood were not seen to show any tendency to form strands.

The microscopic appearance of the hyphae in infected wood of *B. alicastrum* was examined by staining microtome sections in safranin and picro-aniline blue (Cartwright, 1929). At first the hyphae develop mainly in the medullary rays, and it is only in wood blocks exposed to the fungus for more than 4 months that the other elements of the xylem are noticeably attacked. The hyphae penetrate the cell walls both through the pits and by means of bore holes about the same diameter as the hyphae themselves. No clamp connexions have been observed in decayed wood.

The fungus in culture

The following description of the cultural characters of *P. carbonacea* is based entirely upon the appearance of cultures grown on 5 % malt agar. Growth spreads from the transplant as a very symmetrical colony with an advancing front of appressed hyphae. The mycelial mat becomes downy in texture, approaching velvety at temperatures much higher or lower than

the optimum. The culture is at first entirely white, but soon turns grey, and finally light chocolate-brown, particularly when the culture is exposed to light. In old cultures, abortive hemispherical fruiting-bodies may sometimes be formed.

The hyphae, which measure 3–8μ in diameter, are mostly hyaline and bear simple septa. Clamp connexions have been seen in old cultures, but are extremely rare. Branching of the hyphae is dendritic. Encrusted hyphae, and cuticular cells superficially resembling chlamydospores, are characteristic of old cultures.

An extensive key for identifying cultures of wood-rotting fungi has been published by Nobles (1948). This key is based on eleven characters, both macroscopic and microscopic, and is essentially a numerical indexing system for recording the diagnostic features. Since cultural characteristics are particularly important from a taxonomic point of view in the genus *Poria*, the species of which possess comparatively few morphological characters, *P. carbonacea* is keyed out according to Nobles's system in Table 1. This key pattern (1, 2, 2, 3, 0, 2, 2, 2, 1, 1, 1) serves to distinguish *P. carbonacea* from other species which resemble it in external form.

Table 1. *Characterization of* Poria carbonacea *on the basis of Nobles's key* (1948)

Character	Number assigned to *P. carbonacea* according to Nobles's system	Verbal description
Host	1	Occurs on broad-leaved trees
Colour of mycelial mat	2	Mat yellow or brown, at least when mature
Reaction on media containing tannic acid	2	Diffusion zones lacking on media containing tannic acid
Septation of hyphae	3	Hyphae of advancing zone with simple septa, those of older part of mat with clamp connexions
Special structures	0	Encrusted hyphae. N.B. In *P. carbonacea* they are not contorted
Chlamydospores	2	Chlamydospores lacking. N.B. Cuticular cells, superficially resembling chlamydospores, are nevertheless present
Conidia	2	Conidia lacking
Oidia	2	Oidia lacking
Growth rate	1	Rate of growth rapid, plates covered in 1–2 weeks
Fruiting	1	No fruiting before the end of 6 weeks
Effect on agar medium	1	Reverse side of culture brown, at least in part, before the end of 6 weeks

Physiological data

Temperature

Rate of growth at different temperatures was determined by the method described by Cartwright & Findlay (1934). The daily increments (mean of five replicates) in the diameter of Petri dish cultures on 5 % malt agar (Table 2) show that the fungus grows very rapidly at the optimum temperature, which is near 31·5° C. The maximum temperature for growth is

about 38° C., and, although the minimum temperature was not determined, the rate falls off very rapidly below 22·5° C. These figures indicate that *P. carbonacea* may be regarded as a high-temperature fungus, which is consistent with it being a tropical species.

Table 2. *Influence of temperature on the growth of* Poria carbonacea

Temperature (° C.)	20·0	22·5	25·0	28·0	30·0	31·5	32·5	35·0	37·5	39·0
Mean daily increment in diameter (mm.)	12·7	21·4	23·7	28·3	31·6	37·9	34·9	29·6	19·0	Nil

Humidity

The rate of lateral extension of mycelium at different relative humidities was investigated in ground-glass-stoppered jars by Tomkins's (1932) method. The jars were maintained at 30° C. (i.e. near the optimum temperature), and sodium chloride solutions were used to regulate the relative humidity. The results of this experiment are given in Table 3, each figure being derived from the measurement of two replicates. No measurable growth had occurred after 10 days at relative humidities lower than 90 %, but above this level the higher the humidity the faster the spread of mycelium. In practice this result probably means that *P. carbonacea* requires a relatively high water content for growth in wood, and therefore would be sensitive to drying.

Table 3. *Influence of humidity on lateral extension of the mycelium of* Poria carbonacea

Relative humidity (%)	98·7	97·1	95·5	93·6	91·7	88·5	87·0
Mean daily increment in diameter (mm.)	23·5	23·3	18·3	11·1	3·1	Negligible	Nil

Table 4. *Rate of decomposition of wood blocks by* Poria carbonacea

	Mean % loss in dry weight after 4 months at 22° C.
Brosimum alicastrum	10·36
Pinus sylvestris	3·82
Fagus sylvatica	2·68

Rate of decomposition of wood blocks

In view of the rapid growth of *P. carbonacea* on agar, it was decided to determine the rate of decomposition of small blocks of *B. alicastrum* (the only wood that is definitely known to be attacked by this fungus in Jamaica) under laboratory conditions. The method described by Findlay (1938) for testing the natural durability of timbers was followed, except that the test blocks measured $5 \times 2{\cdot}5 \times 1{\cdot}5$ cm. and square household pickle jars were substituted for Kolle culture flasks. Blocks of *Pinus sylvestris* and *Fagus sylvatica* (readily attacked by most wood-rotting fungi) were included in the test for comparison. The tests were made at 22° C., and not at 30° C. as in the humidity experiment, because 22° C. is the standard temperature for performing wood-block tests. The losses (means of five replicates) in dry weight during 4 months' test are listed in Table 4. These results show that, although *Pinus sylvestris* and *Fagus sylvatica* are

resistant to *P. carbonacea*, *B. alicastrum* is very definitely non-resistant. The current assumption among many Jamaican builders that *B. alicastrum*, because it is one of the local hardwoods resistant to termite attack, does not need treatment to protect it against wood-destroying fungi is therefore a fallacy.

Comparison of Poria carbonacea *with other fungi for ability to decay the wood of* Brosimum alicastrum

It is of interest to know how *P. carbonacea* compares with other well recognized, active wood-destroyers in its ability to decay the wood of *B. alicastrum*. For this purpose the following four test fungi were chosen: *Coniophora cerebella* Pers., *Lentinus lepideus* Fr., *Polystictus versicolor* Fr. and *Polystictus sanguineus* Fr., the last because it is a common wood-rotting species in most tropical countries. The same procedure was adopted as in the tests on the rate of decomposition of wood blocks, except that only two test blocks (instead of the recommended ten) were exposed to each of the four test fungi. The test was made at 22° C., because comparative figures on the resistance of other timbers to decay by these test fungi are available for this temperature alone. The losses in dry weight after 4 months (Table 5) show that *Coniophora cerebella* caused the most severe decay but that *P. carbonacea* was about as destructive as the other three fungi tested.

Table 5. *Decomposition of blocks of* Brosimum alicastrum *wood by* Poria carbonacea *and four other fungi*

	Mean % loss in dry weight after 4 months at 22° C.
Poria carbonacea	10·36
Coniophora cerebella	23·77
Lentinus lepideus	10·95
Polystictus versicolor	14·11
P. sanguineus	11·68

We are indebted to Mr Derek Reid for identifying *Poria carbonacea*, to Dr L. Chalk for identifying the infected timber, and to Dr W. P. K. Findlay for supplying wood blocks and fungal cultures.

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(*Accepted for publication* 30 *November* 1959)

REVIEWS

Plant Pathology: Progress and Problems, 1908–1958. Edited by C. S. Holton *et al.* (Published for the American Phytopathological Society by the University of Wisconsin Press, Madison, 1959.) Pp. xix + 588, 39 text-figures and 32 plates. Price U.S. $8.50.

The foundation in 1908 of the American Phytopathological Society was an important event. The Golden Jubilee of that event, celebrated in 1958 by an international gathering of plant pathologists at Bloomington, Indiana, is now shared with a wider audience by the publication of its proceedings reviewing the current status of the science. This handsome volume is a noteworthy exception to the usual assumption (except by publishers) that Conference proceedings are not worth publishing. 'The Golden Treasury', as it has already been named in this country, has been magnificently conceived by its organizers in the form of ten sections on: history and development of plant pathology; physiology of parasitism; genetic mechanisms governing pathogenicity and disease resistance; fungicides; chemistry of fungicides; soil microbiology and root disease fungi; concepts and problems of nematology; structure of viruses; multiplication of plant viruses; and epidemiology of plant diseases. From the opening paean by Stakman to the thoughtful final pages by van der Plank the distinguished authors of the fifty-one contributions gave of their best, and with a fitting sense of the occasion.

Reading this book straight through is a remarkable experience. For the most part it is an exciting book written by excited people, trying to see their own specialisms in perspective against the development of the science of plant pathology during the half-century of progress in which the American Phytopathological Society has played an important part. To review the book adequately is impossible, but some reflexions evoked in a British reader may be relevant. The first impression is one of breadth of choice in selection of contributors from many countries, a selection not confined to the elderly eminent but giving younger men their share. We learn that 42 of the 130 Charter Members of the Society survived to the Jubilee. Yet I estimate that fewer than half the contributors were out of the cradle when the Society was founded. The individual contributions are not only valuable in themselves but also provide a source of 2852 selected references to the literature of plant pathology. Of these references 91·2 % are in the English language, 5·5 % in German, 0·95 % in French, 0·63 % in Dutch, 0·46 % in Japanese, 0·32 % in Russian, and 0·84 % in other languages. Does this mean that the problem of an international language has already been tacitly solved for the science of plant pathology? Or does it mean that the world is divided into almost closed groups for the science of plant pathology, as we suspect it is for the practice of the craft?

Mycology, even Applied Mycology, long ago ceased to be synonymous with Plant Pathology, and problems of taxonomy and nomenclature of the fungi find no place here. With commendable wisdom the organizers of the Jubilee Meeting have concentrated on the experimental achievements of Plant Pathology, though as a result we meet the white-coated scientists but miss the sweat and mud on the field worker. Details of advisory work are naturally out of place in this context, although the U.S. record here is also a proud one. We find little on the measurement of crop loss, less on field survey and assessment. Perhaps more serious still is the selling out to agronomy of plant pathology's interests in yields and crop sequence—here indeed something valuable has been lost in the fragmentation of science. Had the occasion been celebrated in Britain the treatment would have been somewhat different, but we shall not be able to celebrate such an occasion before A.D. 2010. Although our Mycological Society is a generation older than its American counterpart, we have not yet founded a Phytopathological Society to guide our progress. The best we can do is to express our wholehearted thanks to the Americans for theirs.

P. H. GREGORY

An Introduction to Industrial Mycology. By GEORGE SMITH, 5th edition. (London: Edward Arnold (Publishers) Ltd., 1960.) Pp. xvi + 399, 168 text-figures. Price 40*s*.

After the major increase in size of the fourth edition (see these *Transactions*, **37**, 441, 1954) over the third, the latest edition of this well-known and much appreciated text may be considered to represent a phase of consolidation. It has twenty-one more pages and seven more illustrations than its forerunner. The general index has been replaced by separate author and subject indexes and many minor adjustments and additions have been made to a standing type. This increases the book's usefulness and though owners of the fourth edition may feel the expense of a replacement unjustified, the latest edition at its higher price can be recommended as a 'best buy' for anyone having a practical interest in moulds.

G. C. AINSWORTH

NEW CHECK LIST OF BRITISH AGARICS AND BOLETI

ANNOUNCEMENT FOR COUNCIL BY THE HON. TREASURER

The *New Check List of British Agarics and Boleti, Parts* I *and* II was issued as a Supplement to the June, 1960, number of these *Transactions*, to all personal Members of the Society paid-up on the date of publication and to all regular Trade subscribers. Those now wishing to obtain the *New Check List* will have to purchase it at the published price (15*s*. net + postage: $2.50 in the U.S.A.) from the Cambridge University Press (200 Euston Road, London, N.W. 1) or through a bookseller.

The *New Check List* will not be given as a free Supplement to future purchasers of Volume 43 of the *Transactions*.

WALTER BUDDIN

July 1960.

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Guide to the literature for the identification of British fungi. 1952. Price 2*s.* 6*d.* Postage, 4*d.*

Census Catalogue of British Lichens. By W. WATSON. 1953. Price 7*s.* 6*d.* Postage, 5*d.*

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INSTRUCTIONS TO AUTHORS

Contributors are asked to be clear and concise, and to use a recent issue of the *Transactions* as a guide to the arrangement of heading and sub-headings, explanations of plates and figures, references to the literature, and other details. Each paper should start with a short summary. Contributors are urged not to adopt long serial titles and sub-titles.

The standards adopted in the *Transactions* are those outlined in the Royal Society's pamphlet, *General Notes on the Preparation of Scientific Papers* (1950, The Royal Society, Burlington House, London W. 1, price 2*s*. 6*d*.). An exception is that in the *Transactions* citations of references to literature, although based on the Harvard system, include the full titles of the papers and complete page numbers. Titles of journals are abbreviated in accordance with *A World List of Scientific Periodicals*, 1952.

The scientific names of genera and species are printed in italics and should be underlined in the typescript. The title of the paper, headings, and subheadings may be in capitals, but should not be underlined.

The attention of authors proposing new taxonomic groups is drawn to the need to give adequate descriptions and figures, and to designate the types as required by the *International Code of Botanical Nomenclature*. The description of a new species should be accompanied by a figure, and whenever possible the type specimen should be deposited in one of the national herbaria.

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